THE JOURNAL OF AGRICULTURAL SCIENCE

EDITED BY

R. H. BIFFEN, M.A. T. H. MIDDLETON, M.A.

A. D. HALL, M.A. T. B. WOOD, M.A.

IN CONSULTATION WITH

W. BATESON, M.A., F.R.S. J. R. CAMPBELL, B.Sc.

W. SOMERVILLE, M.A., D.Sc.

Volume II. 1907—8

CAMBRIDGE:
at the University Press.

LONDON: Cambridge University Press Warehouse, Fetter Lane
(C. F. CLAY, Manager)
and H. K. LEWIS, Gower Street.
NEW YORK: The Macmillan Company.
LEIPSIC: F. A. Brockhaus.
BERLIN: A. Asher and Co.
BOMBAY AND CALCUTTA: Macmillan and Co., Ltd.
Entered at the New York Post Office as Second Class matter.

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

CONTENTS.

Part 1 (January, 1907).

Part 2 (April, 1907).

Part 3 (December, 1907).

Part 4 (July, 1908).

VOLUME II JANUARY, 1907 PART I

THE IMPROVEMENT OF ENGLISH WHEAT.

A RÉSUMÉ BY

A. E. HUMPHRIES, *President of the Incorporated Association of British and Irish Millers*,

AND

R. H. BIFFEN, M.A., *Agricultural Department, Cambridge University.*

THE following *résumé* of the work so far published by the Home-grown Wheat Committee of the National Association of British and Irish Millers is based upon a paper presented by one of us to the International Convention of Millers which met in Paris during October, 1905[1]. This work being well past the tentative and preliminary stages it proved possible to make a broad survey of it and to piece together isolated special portions which have been published from time to time.

Its publication has two objects in view: one to bring the work to the notice of agriculturists to whom otherwise it might be inaccessible, and the other to persuade those who recognize what results may accrue from a many-sided investigation of this kind to take up the study of our other farm crops on somewhat similar lines. Many will reply that such work may well be left in the hands of the professional seedsmen, but if they will look back upon the history of any one of our farm crops and consider the improvements, or otherwise, made during the last twenty years they will probably arrive at a different conclusion. The story of wheat, *mutatis mutandis*, is much the same as that of many of our crops, and where as in this country large monetary returns per acre are essential the quality of the crops can no longer be neglected.

[1] "The Improvement of English Wheat," by A. E. Humphries, published by the Incorporated National Association of British and Irish Millers, 59, Mark Lane, London, E.C.

Plant-improvement has in the immediate past been too much associated with efforts to obtain greater yield, shapeliness, colour, etc., whilst results of great value are to be won by seeking for that most subtle of attributes, quality.

As far back as the year 1890 the National Association of British and Irish Millers called attention to the fact that the quality of our home-grown wheats had deteriorated, and pointed out that "such wheats bring but a poor price and require a large admixture of strong foreign wheats to make a flour that will bake into a satisfactory loaf of bread." At this period the older varieties, such as Chidham, Rough Chaff, and Lammas, were being supplanted by varieties of the Square Head and Stand-Up types, whilst the growing importation of strong wheats had set up a much higher standard of quality. The appeal circulated by the Association had little or no effect, and as a result of the deterioration of quality and the simultaneous steady decline in the acreage devoted to wheat many of the inland mills went out of existence. In the following years numerous new varieties of wheat were introduced, many of them claiming to possess excellent milling qualities, yet in 1900 the Association found it necessary to again call attention to the fact that the quality of our English wheats was still deteriorating. They attributed this to the fact "that farmers and seed-raisers pay more attention to the large quantity of straw and wheat produced by some of the newer varieties, whilst overlooking the fact that many of these are singularly destitute of gluten and of other characteristics which are of the utmost value for milling purposes." To anyone inspecting the wheats at our agricultural shows it will be clear that this is still the case to-day. The large berry, which is soft and obviously full of starch, is still the judge's ideal.

Efforts were also made by the Millers' Association to induce the most prominent agricultural societies throughout the kingdom to take the matter up, but, though it was all too clear that the English varieties then grown could not compete with the foreign grain coming in ever-increasing quantities into the country, and that consequently the prices obtained by the home producer were and must continue to be unsatisfactory, nothing was done.

This state of affairs was not confined to Britain, but prevailed in other countries in Western Europe. In Denmark, for instance, our square-headed wheats were introduced about 1880 with the result that this same complaint of lack of strength was quickly made. A committee appointed by the Royal Agricultural Society of Denmark began

to investigate the question in 1882 and reported in 1896 that it was not profitable under their conditions to grow wheats of greater strength and pay for it with a smaller yield. If strength and low yield are correlated then it may be that all attempts to obtain strong wheats suitable for our conditions are doomed to failure, but of this assumption there is no proof whatever.

In 1901 the National Association of British and Irish Millers despairing of help from the agricultural societies of the country called into existence a small committee representing both the milling and agricultural industries, supplying it to the best of its ability with funds to investigate the possibilities of improving our home-grown wheats. Since the inception of the work it is pleasant to note that many of the experimental stations both at home and abroad have willingly given their assistance, and that the Board of Agriculture have from time to time provided the committee with additional funds. The ultimate object of the committee's work has been to produce strong varieties of wheat of good yielding power suitable for our English conditions of cultivation, since the varieties now in existence, excellent as they are in many respects, are without exception lacking in the strength which modern standards of bread-making demand.

We must at the outset define "strength." The term has been used in various ways by different authorities with the result that the study of the literature dealing with such problems has become somewhat confused. Many writers, particularly on the Continent, estimate strength by the quantity of water a given quantity of flour will absorb in producing dough of a standard consistency, the consistency being measured by various methods, such as its stretching power, its resistance to puncturing, etc. In practice such methods are unsatisfactory, for the bakers do not make the various kinds of flour up to one and the same consistency in the doughs. To give the best possible loaves some require to be made into "tight," others into "slack" doughs, and the baker simply learns by experience what particular degree of consistency is the most suitable for the flour he has in hand. Another common method of attempting to give some idea of the strength of flour is to associate it with the number of loaves a given quantity of flour (in practice a sack) will produce. The larger the number of the loaves produced the stronger the flour is considered to be. Under such circumstances the suitability of the loaf from the consumer's standpoint, if not from the baker's, is neglected. Some Russian and most Indian wheats, for instance, give a large numerical

yield per sack, but the loaves are small and close in texture. A third view, apparently largely adopted by the bakers, is to judge strength by the way a flour behaves in the doughs, by its toughness, elasticity, freedom from stickiness, etc.; in other words, by the facility with which large masses of dough can be handled in the bakehouse. It seems more satisfactory to regard these as separate characteristics, for though of undoubted importance to the baker they are not necessarily associated with the production of satisfactory loaves. The fact that some of the Russian wheats from St Petersburg or Reval are esteemed strong, but work very badly in the doughs, will show the necessity for this distinction.

The definition finally adopted by the committee is, that a strong wheat is one which yields flour capable of making large, well-piled loaves, the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily. To estimate the strength of any particular sample of wheat then it is necessary to grind it and make the final tests in the bakehouse.

In order to minimize as far as possible the chances of errors involved in baking tests certain precautions have been observed throughout. In the first place the baking trials are made with sufficient flour to yield a batch of about half-a-dozen loaves—the "cottage" shape being considered the most satisfactory. With each set to be tried loaves are baked from flour whose quality has been accurately ascertained. To these standard loaves a certain number of marks are assigned, and by comparison the baker records in marks his opinion of the strength of the flour under test. On this arbitrary scale the strongest wheats in commerce mark about 100, "London Households" 80 to 85, and average English 60 to 65. The tests are always carried out by a man who devotes the whole of his time to this kind of work, and repeated trials have shown that they may be relied upon to express the strength with substantial accuracy.

It was foreseen, however, that this process would be impracticable on a very large scale, if only on account of the labour involved in growing sufficiently large plots of the many varieties of wheat the committee anticipated they would have to deal with. Other methods for estimating strength were therefore sought for.

From time to time various methods have been suggested for this purpose. These have been subjected to a critical examination at Rothamsted[1]. One of the oldest methods assumes that the percentage

[1] Hall, *Journal of the Board of Agriculture*, Vol. xi. p. 321.

of gluten extracted from a flour by washing is an index of its strength. In certain cases it was found that the strength determined by the gluten test and by baking trials agreed, but so many exceptions occurred that it was evident that the method could not be relied upon. It appears to be rather the quality of the gluten than its amount which determines strength. The character of the gluten itself has been invested by Girard and Fleurent, and they have come to the conclusion that the relative proportions of gliadin and glutenin contained in it determine its quality. In their opinion a ratio of 75 of gliadin to 25 of glutenin is the criterion of a good flour.

Guthrie in New South Wales and Snyder in Minnesota also make use of the gliadin-glutenin ratio as a test for strength. The latter considers the ratio of 65 : 35 indicates a well-balanced gluten. The difference between these figures of Fleurent and Snyder is mainly due to differences in the method of estimating gliadin. The value of this test has been open to criticism from the first. On comparing the results obtained by the gliadin-glutenin ratio with those of the bake-house there appears to be "no agreement whatever between them, when flours of widely differing characteristics are being tested."

On the whole the estimation of the total nitrogen percentage in either the grain or the flour gives results which are nearest to agreeing with the baking trials, those wheats with the highest nitrogen content being the strongest. A certain amount of caution has to be used in applying the test, for it is by no means an absolute measure of strength. For instance, the highest nitrogen content in a series of trials with Square-Head's Master grown on different soils was 1·97, and its baking marks 40, whilst a better sample marked at 62 had a lower nitrogen percentage, namely 1·66. Where the wheats are grown under similar conditions the nitrogen content appears to be a satisfactory index of their strength.

One of the first problems attacked was that of the cause of strength in wheat. It is popularly supposed in this country and in many parts of Western Europe that wheats are poor in quality owing to climatic conditions. Here, for instance, it is often stated that lack of sunshine and the poverty of the soil compared with the virgin lands of the wheat-growing districts of Canada and the United States are the controlling factors in making our wheat so deficient in strength. If this were to prove the case then the possibilities of improving the quality of English wheat would be slight, but many facts are known which militate against this view. The fact that India, Australia, and California export wheats

no stronger than our own makes it clear that abundance of sunshine does not necessarily result in the production of strong wheat, and a comparison of the strength of wheat grown in the wet and sunless season of 1903 with that of the sunny season of 1904 showed on the whole a slight difference in strength there in favour of the year with less sunshine. In short it may be affirmed that climatic conditions, such as amount of sunshine, temperature at various stages of growth, rainfall, or the amount of moisture present in the air have no such noteworthy effect on strength that we need despair of growing strong wheats in this country. Investigations on the influence of soil on strength have been carried out in the following manner. Two varieties of wheat, Red Lammas and Square-Head's Master, were grown side by side on seven typical soils. Red Lammas is one of our best varieties, whilst Square-Head's Master is of indifferent quality. The plots were harvested as nearly as possible under the same conditions of ripeness and a portion of the wheat ground and tested in the bakehouse. In the following table the results are expressed by the baker's marks:

Soil	Lammas	Square-Head's Master
Warp	70	62
Deep loam	65	40
Fen	65	55
Sand	62	55
Deep sandy loam	58	42
Thin chalky loam	57	30
Stony clay	50	40
Average	61	46

from which it is evident that although the better samples of Square-Head's Master were superior to the worst samples of Lammas yet on every soil the Lammas proved itself the stronger variety of the two. The table further shows that soil conditions have a considerable influence on the strength of wheat, though it gives no information as to the precise factor or groups of factors determining it. Neither does it follow from these results that warp soil will invariably grow good, and thin, chalky soils poor quality wheat, for in the case of another variety, Fife, this latter soil has produced wheat of really great strength. It will therefore be necessary to find varieties capable of giving the maxi-

mum of strength under strictly local conditions, and this problem each locality may have to solve for itself.

Once it was proved that the soil in some way influences strength, the question arose as to whether it would be possible to secure improvements in this direction by manuring. The wheat plots at Rothamsted and Woburn offered unique facilities for such an investigation, and with the cooperation of the trustees of the Rothamsted Experimental Station and of the Royal Agricultural Society of England, this was commenced. The results obtained up to the present are surprising in many ways. A brief summary of the more important ones are given in the following table:

Rothamsted			1903 crop			1904 crop	
Broadbalk Field Plot No.	Dressings	Yield per acre	Strength Nov. 17, 1903	Strength May 12, 1904	Strength June 28, 1904	Strength Autumn, 1904	Strength Spring, 1905
3	Unmanured	12·8	50	50	55	45	60
10	Amm. Salts = 86 lbs. N.	20·4	3	30	48	20	38
11	,, + Super	23·6	0	30	45	20	45
7	,, ,, + Minerals	32·6	below 0	below 0	—	10	35
2b	Farmyard Manure	35·3	below 0	below 0	below 0	—	—

In both the years 1903 and 1904 the wheat from the unmanured Rothamsted plots was considerably superior in strength to that of the manured plots. Thus after the wet harvest of 1903 in the bakehouse the unmanured plot earned 50 marks, plot 10 with ammonium salts 3, plot 11 with ammonium salts and superphosphate 0, plot 7 with a "complete dressing" below zero, and 2*b* with farmyard manure below zero. In 1904 the corresponding marks for these plots were 45, 20, 20, 10 and below zero. Strangely enough the wheat from the highly manured plots actually appeared to the eye to be stronger than that from the unmanured, and analysis showed that the use of nitrogenous manures had increased the percentage of nitrogen in the grain. Further baking trials brought out the fact that the strength of the grain from the highly manured plots was increasing abnormally with its age. On Nov. 17th, 1903, the unmanured plot earned 50 marks and 55 on June 28th, 1904, or practically its quality was unaltered, whilst plot 10, marked 3 on the former date, had risen gradually to 48 on the latter, when further improvement ceased. The 1904 crop showed the same

result though the differences were not so obvious. This marked improvement in quality on ageing coincided with a deficiency of phosphates in the ash of the grain.

The results obtained on the light soils of Woburn differ radically from those at Rothamsted, although the strength of the wheat on the unmanured plot was practically the same at both stations. Without describing them plot by plot it may simply be stated that the strength was practically unaffected by any form of manuring, and that there was no noteworthy development of it after ageing. The differences in the results are difficult to explain, but the experiments show satisfactorily that long-continued heavy manuring does not increase the strength of wheat, and it may even seriously depreciate it.

Many of the districts producing strong wheat have, owing to the severity of their winters, to resort to the cultivation of rapidly growing types which will mature satisfactorily when spring-sown. This is the case for instance with the wheats of Minnesota, Dakota, Manitoba, and also with Ghirka wheat. This fact may possibly explain the view so generally held in this country that spring-sown wheats are stronger than autumn-sown ones. But that spring-sowing can be an actual source of strength appears to be negatived by the fact that some Russian wheats, such as Azima, are autumn-sown, and that the same is true of Kansas and some of the Hungarian wheats. This question of a possible correlation of strength and rapid maturation has, however, been investigated in some detail, in the first place in the hope of obtaining some information on the factors regulating the production of strong grain, and further because a really satisfactory spring wheat, if found, would be a useful standby to farmers when unsuitable conditions prevailed for autumn planting.

Most of the trials had to be made with foreign wheats owing to the fact that so few of our varieties mature satisfactorily when spring-sown. This proved to be the case with Scholey's Square-Head for instance.

All of the evidence obtained so far tends to show that no tangible increase of strength results from spring-sowing. For example in 1903 ten different sorts of wheat were spring- and autumn-sown on various experimental farms. As some of these sorts were grown at two or more places fifteen distinct pairs of baking trials were possible. In no case did the spring-sown wheat prove appreciably stronger than the autumn-sown, the baking marks being practically level in each case. The average mark for the series was 83 for the autumn-sown and 83 for the spring-sown. A second series of eight sorts grown by one of us

in 1904 gave the same result, the spring- and autumn-sown wheats earning 77 and 79 marks respectively. As 1903 and 1904 were typically wet and dry seasons the results may be taken to hold for a wide range of climatic conditions. A further trial made with Nursery wheat which is one of the few English varieties suitable for the purpose was conducted on slightly different lines. The grain was sown in the middle of November, and again at monthly intervals until April. With the exception of the April-sown plot, which had to be abandoned, a satisfactory plant was obtained in all cases. On testing the five remaining plots the strength was found to be practically the same in all.

In addition to the baking results of these various trials one of us has recorded the number of days from sowing to earing, earing to forming grain, forming grain to ripening, together with the average daily temperatures throughout this period. The mass of statistics, for which the original paper[1] must be consulted, show that under our conditions the importance of rapid maturation has been much overestimated. In 1903 for instance the strongest of the autumn-sown wheats averaged 58 days from earing to ripening, whilst some of the weakest took the same, or even a less, time. A few figures taken from the 1904 trials will show further how little connexion there is between strength and rapid maturation. Three strong wheats marking 92, 87, and 84 respectively matured in 42, 45, and 44 days, and three weak ones marking 67, 63, and 61 in 42, 42, and 40 days. The shortest period between earing and ripening was 38 days, and this particular variety only earned 65 marks in the bakehouse. The weakest wheat in the series marked as 45 took 48 days to mature.

The strength of wheat has for many years been considered to be largely dependent on the time at which it was harvested. If this were the case and no marked depreciation in yield resulted from an early harvest it is clear that it would be advantageous to investigate the most suitable stages at which the crop should be cut. The first case examined was that of Rivet wheat cut when dead ripe, normally ripe, and green. The two latter samples were grown on the same farm, but the first on a different one. On baking, the three samples were marked 15, 35, and 52 respectively. The total nitrogen percentage and the gluten tests also indicated that the material cut green had about the average strength of English wheat, whilst the corresponding figures at the dead-ripe stage pointed to its being greatly deficient in strength. An independent trial gave the same results, the expert reporting that

[1] Humphries, "Improvement of English Wheat."

the dead-ripe sample baked as if the gluten had previously been washed from it. Although this affords strong confirmation of the popular belief in the value of early cutting it was considered advisable to make further trials, so, in the following seasons, samples of different varieties of wheat were obtained from various localities, each set, representing the three stages, being cut from the same piece of wheat. For this trial two sets of Square-Head's Master from different localities, Stand-Up White, and Red Giant were milled. In each case the baking marks for each particular variety showed that there was practically no difference in the strength of the grain at these three stages. There appears to be no reason why the results with the Rivet series should be mistrusted, but as it is desirable to confirm them, experiments are now in hand for this purpose. It should be noted that Rivet wheat belongs to a different sub-species of wheat from those commonly cultivated in this country, and it may be that these marked differences in its strength at various stages of ripening are peculiar to it[1].

From the foregoing account it is clear that although strength can be influenced to a certain extent by the conditions under which wheat is cultivated, the greatest improvements to be obtained by varying these conditions are not likely to be sufficient to bring the strength of even our best varieties to the level of that of such wheats as the Manitoban. It is therefore of great interest to know to what extent the strong foreign wheats can profitably be cultivated in this country, and whether any could be found suitable for our purposes. The work which had already been carried out on the Continent, more particularly that of the Danish Royal Agricultural Society, tended to show that the chief objection to cultivating the strong wheats of other countries was their rapid deterioration under the new conditions, and it did not hold out much promise for the success of such experiments here. Nevertheless the investigation was undertaken by the Committee and is still being pursued. It involves, as anyone familiar with variety-testing will recognize, a great deal of experimental work, but it is a piece of work which the committee are peculiarly suited to carry out, for, being in touch with the National Association of British and Irish

[1] Since this *résumé* was written baking trials have been completed on Fife grown in England, Browick, Garton's New Era, and on three sets of Rivet. The results from the three first named sorts disclose no substantial difference due to the stage of ripeness at cutting. From the sets of Rivet varying results were obtained, so that the only positive statement which can be made on this point is that Rivet is the only sort tried which has shown under any conditions a gain in strength resulting from cutting at an early stage of ripeness.

Millers, and through them with the wheat markets of the world, they are able to obtain numbers of varieties otherwise unobtainable.

The results which have already accrued are full of interest, both to the practical man and the scientist, for whom they open up a number of complex physiological problems. The general plan of the experiments was to test the strength of various wheats as imported, and then sow them as nearly as possible under uniform conditions. Some of the results only can be quoted. One of the first commercial wheats to be tested was that known as Hard Kansas, a wheat, which, unlike the majority met with in commerce, was found to consist practically of one variety only, presumably Turkey, and not a mixture of all the varieties grown in a district. This was grown alongside Carter's Stand Up in 1901. The latter yielded 44 bushels per acre, weighing 64 lbs. per bushel, the Kansas 32 bushels, weighing 67¼ lbs. The following season the yields were about 30 bushels per acre in each case. The bearded ears, the weak straw and extreme susceptibility to rust were all against Kansas from the farmer's point of view, but in the mill it proved as was then considered satisfactory. It was so hard that when ground by itself on a complete roller plant the feed of the mill had to be reduced to give a good "clean up." In the bakehouse it was far superior to average English wheat, and it earned 75 marks. The following season, 1902, its strength was again indicated by 75, but as it fell away to 65 in 1903 the further cultivation was abandoned.

Several of the best of the Hungarian wheats were obtained by Mr Gyorgy, now Minister of Agriculture in Hungary, for testing in the same manner. Two of these, Tisra Videki and Feherinegyei, as imported, proved so extraordinarily strong that marks of over 100 each had to be assigned them. These were grown in 1904, but in spite of the favourable season their strength deteriorated to such an extent that they were little better than ordinary English. The Tisra Videki fell from 120 to 73, and the Feherinegyei from 112 to 77 marks. Two other varieties, Banati and Diozegi, originally marked at 80, retained their strength satisfactorily. In the case of the first two it is clear that the conditions under which they had been grown on the Hungarian plains were largely responsible for these abnormally high marks. Thanks again to the kindness of Mr Gyorgy the committee were able to extend this experiment further by growing Square-Head's Master for two seasons, under the conditions which produced the strong Tisra Videki. A quantity of the grain was sent over here for baking trials, but even without these it was evident that it was simply a good

sample of Square-Head's Master with no abnormal strength about it, and that the climatic conditions had not modified it appreciably. The baker marked it at 70, so confirming this opinion. A supply just received, grown in 1905, for the third year in Hungary, indicates the same result.

Many of the wheats shipped here from Russia are notoriously strong, and in consequence numbers of these have been tested. One unusually good sample, marked 100, consisted of two lots, one grown near Odessa, the other in the Dnieper district, both on light sandy land where the yield was from 12—14 bushels per acre. As grown under English farming conditions the yield went up to 26 bushels, but the crop had nothing to recommend it; the straw was weak and the strength had dropped to 70. Among the many varieties present in the crop were two which were isolated as they appeared to possess unusual strength. One of these is a bearded yellow, the other a bearded red-chaffed wheat; the varieties have still to be properly identified. Pure cultures of these have been raised and sufficient of the yellow-chaffed one obtained for a baking test in which the early opinion as to its strength was justified by its earning 90 marks. Further baking trials of it still have to be made, but after four years' cultivation here it appears to the eye to be as good as when first detected. The red-chaffed one has recently for the first time been baked and it too possesses great strength. In addition its straw and its cropping power are fairly satisfactory, and it may prove worthy of extensive cultivation. Besides these some hundreds of samples of strong Russian wheats have been sown. The majority have proved to be rapidly growing wheats with poor cropping capacity and grain of only medium quality. A few varieties believed to be of more than ordinary strength have, however, been found among them, and these are being kept under observation at present.

The wheats from the north-west of the United States have been tested in the same manner. These again are graded wheats or mixtures of all or nearly all the varieties of a district. On sowing a commercial sample of such a wheat, No. 1 Northern Duluth for instance, several distinct varieties were found in the crop. Some of these varieties multiplied more rapidly than others, so that the composition of the crop from year to year changed and consequently its baking marks would in a very few years have been of no value as indicating whether any of the sorts comprised in the sample were worth further testing. Four of the best varieties were picked out in 1903 and cultivated

since in a pure state, in order to get a sufficient quantity for field trials.

The investigation of the Manitoban wheats has been carried out for the most part on pure varieties instead of commercially-graded seed. In 1902 Dr Saunders sent from the Ottawa Experimental Station quantities of Improved Fife, Preston and Percy. These were grown at Wye and the seed distributed to several agricultural institutions for trial as spring- and autumn-sown wheats. In all these cases the strength of the home-grown progeny was satisfactory, ranging as the result of at least twenty baking tests from 73 to 88 on diverse soils. Of the three it was evident that the Fife was the most suitable for English conditions. Preston and Percy may be more valuable in Canada, where early ripening is of more importance than with us. Further trials both as spring- and autumn-sown wheats have shown that Fife is retaining its strength well, the bakehouse tests marking it from 75 to 89. If only its yielding power were more uniformly satisfactory the cultivation of this variety would go a long way to solving the committee's problem. As it is, Fife has been found to give good crops on some soils. In one case when grown alongside Golden Drop it yielded 43¾ bushels per acre, against 41½ for the latter. Yields of 30 bushels and over have not been unusual in the various trials, but on some lands, more particularly heavy ones, more than 25 bushels cannot be relied on. In the coming season it is being tested by many growers in all parts of England, and it should then be possible to indicate where the cultivation of Fife will prove profitable.

The question now arises as to whether, presuming Fife is suitable for English cultivation, it will be necessary to import seed yearly from Canada, or whether its strength will be retained indefinitely. So far it has been tested for four seasons and there appear no symptoms of any falling away in this respect. Further, there is evidence to show that no deterioration need be expected even under our climatic conditions. Part of this evidence turns on the interesting announcement recently made by Dr Saunders that Fife is none other than Galician wheat, a variety well known in parts of the Continent. Some few berries of Galician found their way in a bulk of seed to David Fife (or Fyfe) in Canada, and proving on account of their superior hardiness to be the sole survivors of the sowing he propagated them further. From these few plants the whole stock of Canadian Fife appears to be descended. Galician wheat can readily be obtained in Western Europe grown under climatic conditions very similar to our own, and

it still appears to have all the strength of its Canadian descendants. Another unlooked for proof that its strength is not a diminishing quantity has been found. Mr Goodwin, of Kidderminster, informed the committee that in his neighbourhood a wheat locally known as Cook's Wonder was being grown which he believed to be Fife, or some closely related variety. He provided them with four samples, grown on widely different types of soil, which on baking proved to be really strong. On inquiring further into the matter it was found that this wheat had been brought over from Canada in 1892 and that no further importation had been made. It has therefore retained its characteristic strength under our climatic conditions for thirteen seasons.

If one must summarize the most important facts brought out in these extensive variety trials the first is that while the quality of some wheats may change enormously with climatic and soil conditions, there are other varieties in cultivation which retain their strength under all conditions. Fife, for instance, is strong whether grown in England, Canada, the United States, W. Europe, or Australia.

It is a peculiarity of certain wheats, such as Fife, to retain this characteristic, whilst others depreciate. So far there are no means of telling how a variety will behave without actually cultivating it, and resorting to either the miller's judgment or the bakehouse. Next in importance is the fact afforded by the series of Lammas and Square-Head's Master trials, that given two varieties of unequal strength, no matter what the soil conditions are, the one which proves the stronger on one soil will prove the stronger on all.

Now that the proof has been given, more definitely than could have been hoped for considering the time the experiments have been in progress, that there is no difficulty in growing strong wheats in this country this knowledge has to be used to the fullest advantage. If the cultivation of Fife wheat, as is probable, should prove unprofitable in many situations, what substitute can be offered for it? Of all the pure strains, now isolated and under observation, numbering nearly a hundred, it would be rash to predict that any are absolutely suitable for extended cultivation. Some possibly surpassing Fife in strength give no promise of a paying yield, others which crop better have thin reed-like straws, and many of them are so extraordinarily susceptible to the attacks of yellow rust that it is problematical whether they would not die out altogether under our conditions.

The attempts made to solve the original problem of combining high-

yield with strength have to be described now that it has clearly come within the bounds of possibility. To achieve this two methods have been resorted to, one the process described by plant breeders as selection, the other hybridizing. In the case of the former the best plants with regard to yield and strength have been chosen from a pure culture of one of the strong varieties, so that strength instead of yield was assured from the first. A portion of the grain from the best five or so of these has been sown in order to obtain twenty-five plants descended from each, and from the small plot producing the best crop the best plants are again chosen, and the process repeated. The variability of the yield appears to be very slight, for after four seasons no manifest improvement has resulted. The attempt to select a strong wheat from such a high-yielding wheat as Rivet hardly appears to be worth making for its endosperm characters are singularly constant. Such an experiment is, of course, not a final one, and it might well be carried out on a larger scale at stations having greater facilities for such trials than are at the disposal of the committee. The data obtained would be valuable, especially as the chances of complications arising through vicinism are remote. The hybridizing experiments however offer far more chance of success in a comparatively short time. These were commenced in 1901[1], and the task of investigating the inheritance of all the numerous characters shown by the different varieties of wheats was undertaken. This seemed essential as it was clear even then that a considerable number of wheat varieties would have to be crossed together. As already stated, the many varieties which have been selected for strength are frequently far from satisfactory in other respects. By applying the experience gained from these preliminary breeding experiments new varieties can be built up in which the objectionable features are wanting, whilst satisfactory ones are retained. At present there is, and naturally so, a great deal of scepticism as to the fixity of such hybrid varieties, and under these circumstances it is satisfactory to be able to state that where the hybrids already raised have been grown in plots containing several thousand individuals they have proved as true to type, level, and uniform in ripening as the oldest varieties in cultivation. The difficulty of obtaining fixed types without endless "rogueing" has practically disappeared.

To build up varieties of wheat suitable both to farmer and miller it was essential to study in great detail the inheritance of strength. The

[1] Biffen, *Journal of Agricultural Science*, Vol. I. No. 1.

matter was complicated from the beginning by the fact that in some wheats the quality deteriorated rapidly, and also by the fact that seed could rarely be spared for analysis during the critical first and second generations. In consequence the inheritance of this characteristic has been judged by eye: that this is fairly satisfactory is shown by the fact that an expert miller when given the grain of two hybrid varieties was able to recognize in them the characters of their parents, although he had no previous knowledge of their history. By the time the third generation is reached and the hybrid varieties are known to be fixed small quantities of the grain can be spared for analysis.

It would be premature to state without any reservation that strength and "weakness" form a pair of Mendelian characteristics, but the assumption that they are so has proved a very valuable one in building up desirable varieties. Each season a number of the best of the strong varieties already selected have been crossed with suitable high-yielding parents, and a number of series of hybrid varieties are being raised. Ruthless selection is practised, and unless wheats prove satisfactory in all features they are destroyed at once, exceptions only being made where they appear of possible value as fresh parents. So far about 40 types for the most part of Fife parentage have survived the ordeal, and these are now being cultivated in the open to determine their yielding-power. They are somewhat diverse in habit, some being loose, others square in the head, some with white, others with red grain, etc., but in each case, as far as the eye can judge, the strength of the parent Fife has been maintained.

THE LAW OF SEQUENCE IN THE YIELD OF WHEAT FOR EASTERN ENGLAND. 1885—1905[1].

By W. N. SHAW, Sc.D., F.R.S.,
Director of the Meteorological Office, London.

In February 1905 I made a short communication to the Royal Society of London (*Proceedings*, Vol. LXXIV. page 562), upon an apparent relation between the yield of wheat for England in the 21 successive years 1884 to 1904 and the aggregate rainfall for the three autumnal months (September, October, November) of the preceding years of the principal wheat-producing districts of Great Britain. The relation was put into the algebraical form:

$$\left.\begin{array}{l}\text{Yield of wheat for}\\ \text{England, per acre}\end{array}\right\} = 39{\cdot}5 \text{ bushels} - \tfrac{5}{4}\left\{\begin{array}{c}\text{previous autumn rain-}\\ \text{fall in inches}\end{array}\right\} \quad (1).$$

In considering the application of the formula to the computation of the yield for the 21 years specified, 7 years were found to give large differences, and reasons were given for regarding the 7 years as being otherwise anomalous.

An endeavour to pursue the investigation of this suggestive relation between yield of wheat and autumn rainfall has disclosed a curious relation between the numbers representing the yield of wheat for a selected part of England, during the last 21 years. This relation is irrespective of any connexion with the previous autumn rainfall, but as it was disclosed in the course of the investigation of that connexion and may ultimately prove to be associated therewith, I will set out the figures first from that point of view.

[1] The earlier part of this paper is a reproduction with slight modifications of a paper on "The Law of Sequence in the Yield of Wheat for Eastern England, 1885–1904" contributed by the author to the "Hann Band" of the *Meteorologische Zeitschrift.* Vieweg und Sohn, Braunschweig, 1906.

The formula already referred to in the *Proceedings* of the Royal Society was based on figures against which objection might be raised, on the ground that they were not strictly correlative. The wheat values were the averages for England; the rain values the averages for those districts of Great Britain where wheat is grown in considerable quantity. It is not easy to obtain figures which refer to an area sufficiently large to eliminate accidental local influences and are, at the same time, properly correlative; but I endeavoured to obtain correlative figures by taking the rainfall values for the district "England East" of the *Weekly Weather Report* and compiling the corresponding values of the yield of wheat from the results given in the official returns of the Board of Agriculture for the counties included in the district.

These figures are, so far as I know, quite unexceptionable for the purpose of the comparison in view. They are doubtless subject to incidental errors, but both on the side of rainfall and on that of yield they are homogeneous for the period to which they refer. It is a matter of regret that they do not extend beyond twenty-one years, the limit set by the wheat returns. The figures for rainfall and for yield of wheat are given in a table at the end of this paper.

The subsisting relation between the autumn rainfall and the wheat-yield of the subsequent year is sufficiently evident, though the constants are different from those in the equation (1) already quoted. For the Eastern Counties the variation of yield in the twenty years is from 25·2 bushels per acre in 1893 and 1904 to 36·3 in 1898, and the formula

$$W = 46 - 2{\cdot}2\,R \qquad (2)$$

gives the yield within the limit of accuracy of 2·1 bushels per acre for thirteen years out of the twenty-one including 1905; but there are eight exceptional years when the yield computed by the formula differs from the actual yield by more than 2·1 bushels. The exceptional years are not all the same as those which were exceptional in the comparison for the whole of England, and on two occasions in particular, viz. 1886 and 1903, the differences, one in excess, the other in defect, are very great.

In the previous comparison an explanation of the differences in exceptional years was given that was *primâ facie* reasonable, and I examined the figures for the East of England in the hope of finding a similar explanation for the differences. In such an enquiry one naturally regards the rainfall data as fundamental, and looks for an explanation of the deficient or too abundant harvests in the accidents that may occur to the crops from floods, hailstorms and other causes,

because the damage done by such accidents bears no numerical proportion to the quantity registered by a rain-gauge. When I plotted diagrams representing the two quantities, rainfall and yield, I expected to find marked irregularities in the yield diagram, with no counterpart in the rain diagram; and I was, for that reason, the more surprised to find that the yield diagram exhibited a singular regularity of sequence, while it was the rain curve that had what may be called abnormal irregularities.

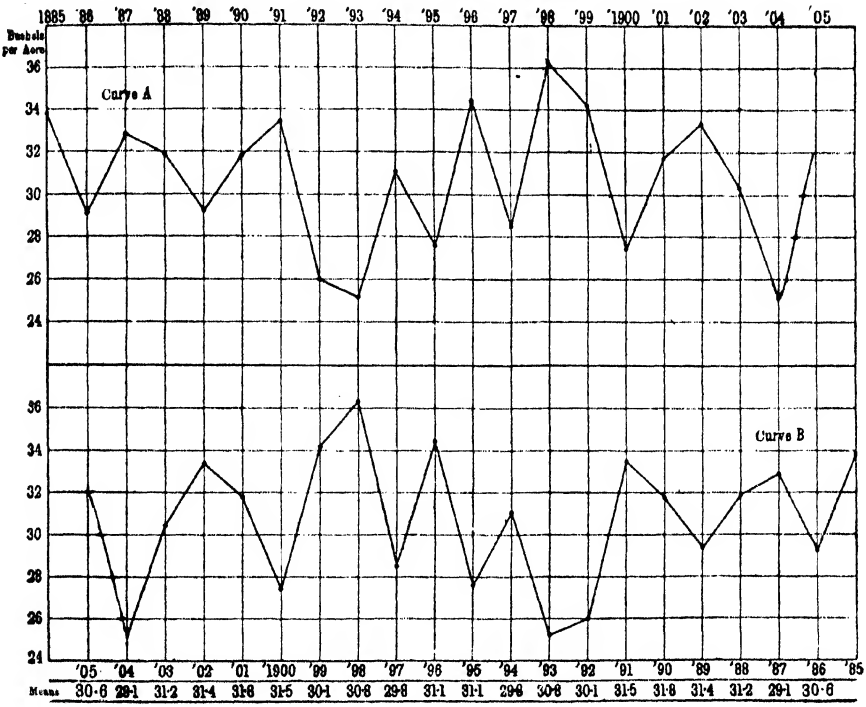


FIG. 1.

The most singular point about the regularity of sequence of the yield diagram is that the curve is almost perfectly reversible with reference to the epoch 1895–6. The average yield for the two years, 1895, 1896, is (31·1 bushels) almost exactly the same as the average yield for the twenty-one years (30·9 bushels); thus 1896 compensates for 1895, and in the same way 1897 compensates for 1894, 1898 for 1893, and so on, without any really considerable exception throughout the twenty-one

years. The worst case of all, an exceptionally bad one, is that for 1887–1904, which, however, gives a mean differing by less than 2 bushels from the average for the twenty-one years.

Figure 1 represents this curious coincidence. Curve *A* shows the yield of wheat for England East in successive years, and curve *B* shows the yield *for the same years taken in the reversed order.* It will be seen that curve *B* is very similar to the simple reflexion of curve *A* from the base line. The numbers at the foot of the figure show the mean value of the ordinates of the two curves in bushels per acre. If the curve *B* were a perfect reflexion of curve *A*, all the mean values would be identical, and it will be seen that they are very nearly so. They range between 29·1 for the pair of years 1887–1904, and 31·8 for the pair of years 1890–1901. The average for the whole twenty-one years is 30·9, so that the greatest difference of the mean of any of the pairs of years from the average of the twenty-one years is 1·8 bushels per acre in the one direction, and 0·9 in the other.

The reversal of the curves can be more strikingly exhibited if one of them be traced and the trace turned round and superposed on the original.

It seems scarcely possible that this compensation, persisting through so many years, should be fortuitous. It would be accounted for if the figures representing the yield of wheat were points on a periodic curve of complex periodicity such that a number of important components concurred in a nodal point in the epoch 1895–6. The test for a nodal point between 1895 and 1896 would be that the ordinates should be reversed in the way in which they are very approximately reversed in actual fact.

It is surprising that this evidence of periodicity exhibits itself in the wheat values, while it is certainly not conspicuous in the autumnal rainfall. The years that are exceptional as regards the rule of parallelism between the autumn rainfall and yield of wheat, viz. 1886, 1889, 1896, 1897, 1901, 1903, and 1905 do not appear as exceptions to the rule of reversal of the wheat yield with reference to 1895–6. The real value for the yield in 1903 is 6·2 bushels below the amount calculated from the autumn rainfall formula, but it compensates the yield for 1888 quite satisfactorily. The only year which shows considerable divergence from the rule of compensation is 1904, which agrees very well with the rainfall formula, but has too small a yield to compensate 1887. It will be seen from the sequel that the defect may probably be attributed to the 1887 yield.

Assuming from the reversal with reference to the epoch 1895–6 that the yield can be represented by a series of periodic components concurring in a node at that epoch, I have endeavoured by an examination of the figures to determine the period and amplitudes of the component oscillations.

There is, so far as I am aware, no organised method of doing this, except for the case of periodic variations of known period with harmonic components and a large number of ordinates for a complete period. In the present case the fundamental period could not be regarded as known. A careful examination seemed to show that the curve might contain components of 2 years', $3\frac{1}{2}$ years', and 11 years' period, and the combination would then only recur completely in 154 years.

To deal with complex periodic variations of unknown period we can proceed by the addition of ordinates with a fixed interval, and so eliminate variations of a definite period [1].

Thus if one of the component variations be

$$a_n \sin \frac{2\pi}{n} t,$$

where n is the period in years, t the time in years from a node, adding the ordinates with an interval of m years gives for this component in the secondary curve the sum,

$$a_n \sin \frac{2\pi}{n} t + a_n \sin \frac{2\pi}{n} (t + m),$$

and therefore a resultant of the same period,

$$2a_n \cos \frac{\pi m}{n} \sin \frac{2\pi}{n} \left(t + \frac{m}{2}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(3).$$

The amplitude is zero if $n = 2m$, and thus, in a curve of complex periodicity, the component $n(2m)$ is eliminated by the addition of ordinates with intervals of m years, while the amplitudes of all the other components are altered by the factor $2 \cos \frac{\pi m}{n}$; the phase of each is put forward by $\frac{m}{2}$ years.

This process has been applied to the curve of wheat-yield by taking the mean of *consecutive ordinates* whereby the variation of two years'

[1] I owe this method of dealing with complex periodic variations to Professor G. Chrystal of Edinburgh, who uses it for the discussion of the complex oscillations of the water of lakes under the name of the method of residuation.

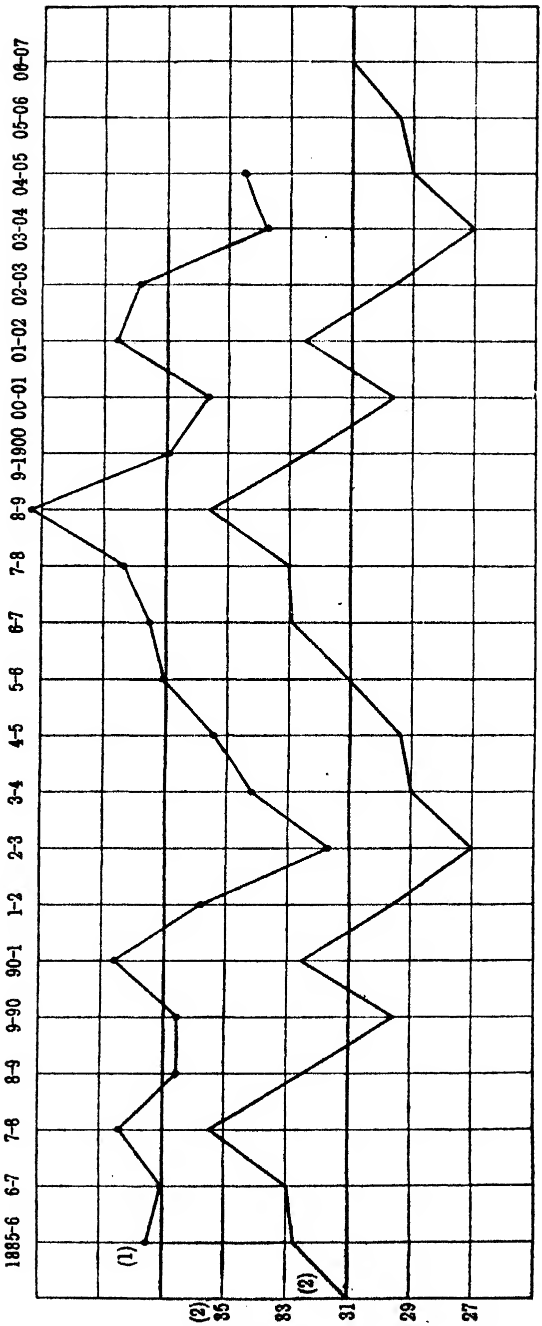

1885-6
6-7
7-8
8-9
9-90
90-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-1900
00-01
01-02
02-03
03-04
04-05
05-06
06-07
(1)
(2)
(2)
35
33
31
29
27

Fig. 2.

period is eliminated; the variations of other periods are put forward in phase by half a year, and the amplitudes are all altered as indicated by the coefficient. The result, which is represented in the upper curve (1) of Figure 2, shows a very considerable simplification of the original curve of Figure 1.

Further procedure by this process is not practicable for two reasons. First, we have only a single point for any year and no means of satisfactory interpolation. We can, therefore, only eliminate components of an even number of years by addition of ordinates. Secondly, if there be any accidental errors in the curve they get distributed over the sum in such a manner as to add to the complexity. There is a considerable reduction of the variation of ordinates upon taking the means of the ordinates (1) + (6) and also of (1) + (7), which points to a component of about ten or twelve years' period. Taking this variation I have endeavoured to guess the components of curve (1), Figure 2, by an inspection of the curve itself.

I have obtained in this way the periodic curve from which the ordinates of Curve (2) are taken and of which the equation is:

$$y = 31 + 2{\cdot}8 \sin \frac{2\pi}{11} n + 0{\cdot}4 \sin \frac{4\pi}{11} n - 1{\cdot}2 \sin \frac{6\pi}{11} n + 1{\cdot}2 \sin \frac{8\pi}{11} n,$$

where n is the number of years (positive or negative) from the node at 1895-6. The successive ordinates correspond with the substitution of $n = 1, 2 \ldots$ in this equation.

The agreement between the curve constructed from this formula (Curve 2) and the original (Curve 1) representing two years' means of actual yield is remarkable. For the first four points the calculated yields certainly exceed the observed, and by an amount which reaches 3 bushels in 1887–8, but from 1889–90 to 1904–5 the mean value of the deviation between observed and calculated values, without regard to sign, is only ·67 bushels, and the maximum deviation + 2·3 bushels for 1902–3. The mean difference between the two curves is 0·1 bushel per acre. A shift of the curve through the space representing ·1 bushel would, therefore, slightly improve the correspondence. The representation of the real curve by the calculated one is, however, already as complete as one can expect any calculation of this sort to be, and pending the compilation of further data we may consider the average yield of wheat for successive two years to be effectively represented by a simple periodic variation of eleven years, with subsidiary harmonic components of 11/2, 11/3, and 11/4 years.

The original article in the Hann volume continued as follows:—

"It is evident that if this representation is accurate the average yield for years to come should fall in with the same formula; in other words the formula allows us to predict the yield of future years. The prediction for 1905 on this basis is a yield that will give with 25·2, the yield for 1904, a mean for the two years of 29 bushels. In other words, the yield for the Eastern Counties for 1905 should be 32·8 bushels. The rainfall calculation for the same year gives 37·6 bushels. There is no doubt that the yield is large, but the Official Report has not yet been issued, so the precise figures cannot be given. If 37·6 proves to be the correct figure there will be rather a large error (2·6 bushels) in the calculated harmonic curve."

The actual figure for 1905 turns out to be 32·0, so that it agrees better with the continuation of the composite curve than with the calculation from the autumn rainfall.

Since the article for the Hann volume was completed, I have extended the computation so as to represent the sequence of the yields for individual years by the sum of a series of six harmonic components of the eleven years' period. This extension of the reasoning accounts for the two year oscillation (which was eliminated from Fig. 2 by taking the average of consecutive years) and regards it as a 'beat' between the oscillations of the fifth and sixth components of the eleven years' period. According to this analysis, of which the details are given in a paper read before the Royal Society on May 17, 1906, the sequence of individual yields for the twenty-one years, 1885–1905, is represented with remarkable fidelity as the sum of six harmonic components of the eleven years' period, each with a node at the epoch 1895–6. The amplitudes of the several components are as follows:

Period............		11	$\frac{11}{2}$	$\frac{11}{3}$	$\frac{11}{4}$	$\frac{11}{5}$	$\frac{11}{6}$ years.
Amplitude	[31]	+ 2·9	+ 0·5	− 1·8	+ 2·8	+ 1	+ 1 bushel per acre.

The − sign indicates a descending node.

Values derived from the addition of these six components are given in the second column of the Table on p. 28, so that they may be compared with the actual values given in the third column. It will be seen that the direction of change from year to year is correctly given in every case and the agreement between the computed and the actual values is very close, except for the years 1887 and 1888, when the actual yield is too small and 1903 when it is too large. The way in which the minor fluctuations in the yield are indicated in the computed curve is very remarkable when it is considered that the components all refer

to an eleven years' period or its submultiples, and the comparison extends over 21 years and not merely eleven years.

If the representation of the actual values by the computed curve have any real counterpart in the law of sequence of the yield of wheat it follows that the yield should repeat itself after eleven years. This is a result which is easily tested. In arranging the Table on p. 28, I have put down not the actual yields but the excess or defect, + or −, from 30·8 bushels, and have grouped together the pairs of years with eleven years' interval; thus 1885 and 1896 are together, 1886 and 1897, and so on. The comparison between any year and the one eleven years later can thus be easily made. For three of the pairs of years out of the ten represented in the Table the yield is repeated within a tenth of a bushel, for six it is repeated within a bushel. In every one of the ten pairs the yields are either both in excess or both in defect. The three cases where there is a substantial difference between the yields for a pair of years eleven years apart are those which contain the years 1887, 1888 and 1903 already mentioned.

That this simple principle of repetition after eleven years, which is easily traceable in the curves of Fig. 1, should not have been noticed until after a somewhat lengthy discussion is due principally to the fact that the yields for 1887 and 1888 are both substantially smaller than the computed values, and the curve, which should have had a salient point then, as it has in 1898 and 1899, has no conspicuous prominence, although its shape is not dissimilar in detail from the corresponding part eleven years later. It is curious, however, that in both the years named the yield, although too small from the point of view just mentioned, exceeded that computed from the previous autumn rainfall, so that the explanation of the defect, if indeed there be one discoverable, is rather recondite. The point is interesting because we have again in the current year a case in which the indication from the autumn rainfall is in the opposite direction to that from the principle of repetition after eleven years, and we do not yet know how it will be resolved[1]. It raises the question of the real meaning of the relation between the autumn rainfall and the wheat-yield of the subsequent year.

A paper by Mr E. Mawley (*Quarterly Journal Roy. Met. Soc.*, Vol. XXIV. p. 75, 1898) "On weather Influences on Farm and Garden Crops" suggested to me that a small autumn rainfall might be associated with a small summer rainfall following, and might therefore become an index

[1] **NOTE. December, 1906. The official estimates for the several counties are not yet published. The general opinion is in that the yield was large but irregular as regards the Eastern counties.**

of general meteorological conditions rather than itself a determining cause of the amount of the future crop. To illustrate this point I have included in the Table on p. 28 in the same line with the figures for the crop for each year the excess or defect from the average of 20 years of the rainfall of each season, from the autumn before to the summer of the gathering of the crop. I have also added as an index of temperature-conditions the "accumulated temperature above 42° F." for the same seasons. The figures are taken from a paper on "The seasons in the British Isles" in the *Journal of the Royal Statistical Society*, Vol. LXVIII. Part II. (June 1905).

Then at the foot of the Table, following Mr Mawley's plan, I have given the average results for the five *best* wheat years and the five *worst.* The result is remarkable. Not only is the autumn rainfall deficient for the good years and excessive for the bad, but, with the exception of the accumulated temperature for the summer, which is above the average in both cases, the character of the season for the good years is the opposite of the corresponding season for the bad years in every case. Judging by the average results for the five years a good wheat yield is preceded by a dry and warm autumn, a rather dry and warm winter, a rather wet and cold spring, and a dry and slightly warm summer, while a bad wheat year has, on the other hand, a wet autumn of average temperature, a wet and cold winter, a dry and warm spring and a moist warm summer.

This bears out the suggestion that the autumn rainfall is in a way the key to the subsequent seasons, but it still remains to consider whether these results are merely average results for the five selected years or whether they apply to individual years. Looking down the figures for the individual years it is clear from the juxtaposition of the signs that a wet autumn usually means a deficient crop and *vice versa*; but it is also clear that a wet autumn is usually associated with a relatively dry spring and *vice versa,* as is indicated in the averages for the five years. On sixteen occasions out of the twenty-one a deficient autumn rainfall has been followed by excess of rainfall in the spring or *vice versa.* On three other occasions the spring rainfall has been normal or within a tenth of an inch of it, leaving only two exceptional occasions, forming the pair 1886 and 1897, when the heavy autumn rainfall was succeeded by spring rainfall above the average. On the other hand, on sixteen occasions out of the twenty-one the deviation of the winter rainfall from the normal has been in the same direction as that of the autumn rainfall. There appears to be no numerical relation between the amounts of excess or defect in either

case, but it does appear that the suggestion of the five year averages that a dry autumn is followed by at least a rather dry winter and a rather wet spring and *vice versa* is borne out in the large majority of cases.

As regards summer rainfall or accumulated temperature, however, there is little information to be got from the figures. Good wheat years are as a rule preceded by warm winters, and bad years by cold ones, but the figures are very irregular. Such a pair of years as 1888, 1899, as a glance at the figures will show, seems to make any generalisation about the relation of the temperature to the wheat or the other elements hopeless from the first.

There are, however, sufficient indications of underlying connexions to encourage further investigation. The eleven years' periodicity in the yield, with its consequence, the repetition of values after eleven years, seems to be the most directly applicable of them. It would of course be unwise to regard the law of sequence thus indicated as definitely established.

The data available give us only approximately two eleven year periods, so that we are not able to apply an adequate test to the suggestion that the curve is in reality a curve of eleven years' period with its harmonic components. Many circumstances besides those associated with the practice of farming might be found to interfere with its persistence. It is possible that the concurrence in a node at the epoch 1895–6 is not mathematically strict; it is possible that the components of about four years', three years', or two years' period are not, strictly speaking, harmonics of the eleven year period. They may disclose increasing divergencies as the years accumulate. All that we can say is that for the twenty-one years for which data exist the representation of the values by a curve of eleven years' period is singularly close to reality, especially for the years 1889 to 1905. Further data, or perhaps data for some other quantity than wheat, may disclose a more definite result. In the meantime it is worthy of note that on the average for the seven counties comprised within England East, the good wheat years have occurred with the following intervals from 1885[1] (itself a good year)

2 years,	1 year,	2 years,	1 year,	3 years,	2 years
1887*	1888*	1890	1891*	1894	1896*

completing eleven years, and then again

2 years,	1 year,	2 years,	1 year,	3 years
1898*	1899*	1901	1902*	1905

[1] The years marked * are those which according to the computed curve give yields exceeding the average by at least 2 bushels; for the others the theoretical yield is not much above the average.

As this order of succession has now occurred twice, it seems worth while to consider the question in relation to the rotation of crops.

TABLE.

Year	Yield above or below 30·8 bushels per acre computed from six harmonic components of eleven years' period.	Actual Yield above or below 30·8 bushels per acre.	Rainfall in inches above or below the average of the 20 years 1881–1900				Accumulated Temperature above 42° F. in day degrees above or below the average of 20 years, 1881–1900			
			Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1885	+4·5	+3·0	-0·3	+1·3	+1·0	-3·2	+25	+46	-104	-89
1896	+4·5	+3·6	-0·9	-1·9	0·2	1·4	+113	-4	+33	+63
1886	-0·7	-1·6	+5·0	-0·6	+0·6	-0·9	-146	-86	-49	-60
1897	-0·7	-2·3	+1·9	+2·5	+0·1	-0·9	-72	-25	+14	+110
1887	+4·8	+2·1	-0·7	+0·3	+0·2	-3·0	+221	-49	-116	+163
1898	+4·8	+5·5	2·5	-1·2	+0·5	-1·6	-58	+71	-68	+2
1888	+4·7	+1·1	-0·9	-1·7	+0·4	+3·4	-244	-81	-87	-213
1899	+4·7	+3·1	-2·4	-0·4	+0·7	-2·8	+244	+139	-21	+201
1889	-2·0	-1·5	-2·2	-1·6	+2·0	+1·8	-40	-46	+67	-25
1900	-2·0	-3·3	+0·2	+3·0	-1·2	+0·2	+99	-36	102	+167
1890	+0·2	+1·0	-0·7	-0·7	0·0	+1·5	-68	-1	+5	-119
1901	+0·2	+1·0	-2·8	-0·4	+0·2	-2·7	+112	+43	-45	+71
1891	+2·4	+2·7	-2·0	-2·6	+1·0	+1·3	+122	-9	-143	-96
1902	+2·4	+2·6	-2·8	+0·4	+0·4	+0·9	+79	+8	-51	-121
1892	-4·3	-4·8	+0·6	-0·2	-0·3	+1·9	+56	-20	+35	-147
1903	-4·3	-0·4	-3·3	-0·8	+0·5	+5·0	+37	+128	+34	-85
1893	-4·4	-5·6	+2·6	+0·6	-3·7	-0·6	-153	-28	+260	+179
1904	-4·4	-5·6	+1·4	+0·7	-1·0	-1·6	+19	-76	+26	+91
1894	+1·1	+0·3	-0·7	-0·5	+0·8	+1·1	-28	+44	+36	-67
1905	+1·1	+1·2	-3·8	-1·4	0·0	-1·2	-6	-11	+19	+187
1895	-4·1	-3·1	+0·3	+0·4	-0·7	+1·4	-27	-69	+62	+15
1906	-4·1	?	-1·6	+1·2	?	?	-127	-20	?	?
Five best wheat years		+3·6	-2·2	-0·8	+0·6	-1·5	+89	+49	-61	+16
Five worst wheat years		-2·5	+1·0	+0·9	-1·4	+0·3	-1	-46	+56	+85

NOTE ON AN APPARENT SECULAR CHANGE IN THE ROTHAMSTED DRAIN GAUGES.

By EDWARD J. RUSSELL, D.Sc. (Lond.),
South-Eastern Agricultural College, Wye.

Dr Miller's interesting and important paper in the last number of this *Journal* (Vol. I. p. 377) raises several points in connexion with the long-continued action of rain on uncultivated and uncropped soils.

Some of the water falling on the drain gauge is retained by the soil, some evaporates, and the rest percolates, but over a series of years the amount of percolation is correlated mainly with the amount of evaporation, when the latter is high the former is low, and *vice versâ.*

Evaporation takes place mainly, but not entirely, from the surface of the soil. A certain amount must go on from the individual particles in the interior of the soil, and the water vapour will diffuse out from the pore spaces at a rate greater than that at which air diffuses in. Other things being equal, the greater the depth to which air can penetrate and from which water vapour can pass out by diffusion, the greater the amount of evaporation. Thus we should expect the greatest evaporation from the 60 inch gauge, a smaller amount from the 40 inch gauge, and the least from the 20 inch gauge.

Examination of the figures for the first four years shows that this expectation is realised.

Table 1.

Evaporation for period 1870–1874.

20 inch gauge	40 inch gauge	60 inch gauge
69·51	71·04	78·90

Calculated as percentages on the evaporation from the 60 inch gauge,

88·10	90·05	100

For subsequent years, however, the relationship does not hold.

The total amounts of evaporation in consecutive seven year periods are set out below.

TABLE 2.

Evaporation during 7 year periods.

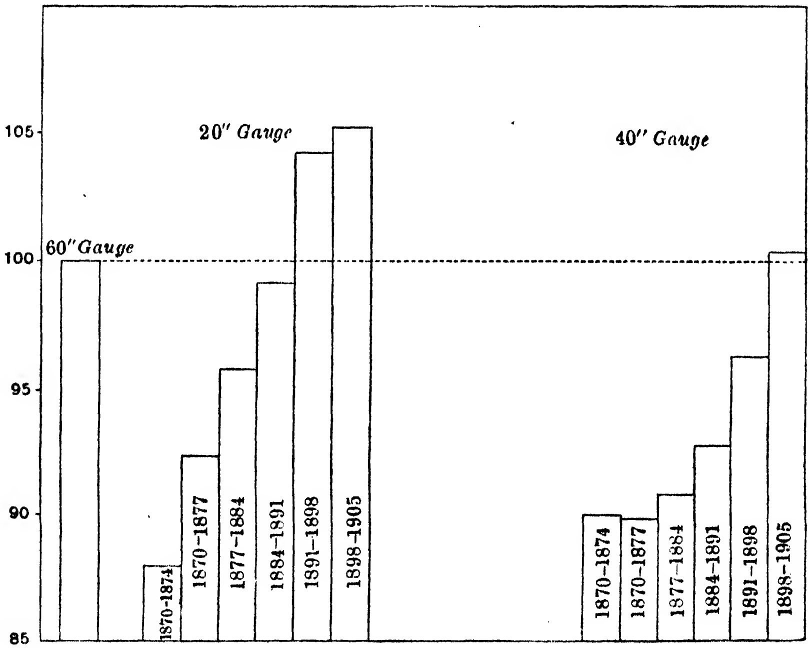
	20 inch gauge	40 inch gauge	60 inch gauge
1870–1877	125·74	122·25	135·81
1877–1884	107·70	102·12	112·39
1884–1891	98·30	92·01	99·10
1891–1898	98·76	91·26	94·80
1898–1905	99·24	94·79	94·23

Calculated as percentages on the evaporation from the 60 inch gauge,

	20 inch gauge	40 inch gauge	60 inch gauge
1st 4 years, 1870–1874	88·10	90·05	100
1st 7 years, 1870–1877	92·60	90·02	100
1877–1884	95·85	90·86	100
1884–1891	99·20	92·85	100
1891–1898	104·2	96·27	100
1898–1905	105·3	100·6	100

The percentage results are plotted in the accompanying diagram.

Evaporation from the Drain Gauges, 7 year periods.



It is quite evident that the relationship between the amounts of evaporation from the three gauges is not constant, but shows a regular and progressive change. At first evaporation from the 20 inch gauge

is lowest; it increases, and soon becomes greater than that from the 40 inch, after about 20 years it equals, and finally exceeds, that from the 60 inch gauge. The 40 inch gauge shows the same change relative to the 60 inch, but with a lag of about 14 years. Up till about 1884 no change took place, and the two gauges behaved precisely as, on the above reasoning, one would expect them to do. Then the relative evaporation from the 40 inch gauge began to increase, and during the last 7 years the amount of evaporation equalled that from the 60 inch gauge. It seems safe to predict that in a few years' time the 40 inch gauge will lose more by evaporation than the 60 inch.

Several causes may operate in bringing about this change.

Rain tends to wash the finest particles downwards into the soil. In pasture land this tendency is counteracted by worms and in arable land by cultivation, but in the drain gauge neither of these factors comes into play, the finest particles wash downwards unchecked, and in time may wash out of the gauge altogether. As they disappear the soil becomes more and more permeable to air and water vapour, evaporation can take place to a greater extent than before, the gauge tends to become dryer, and when rain comes, more of the water is held back and less percolates. Naturally this change is seen in the 20 inch gauge long before it appears in the deeper ones.

It may be objected that the Rothamsted soils contain about 20 per cent. of clay, and the three tons of water falling annually upon the gauges could not possibly wash out anything like this amount. We are, however, not concerned with the total clay but only with the unflocculated portion, which though unknown, is probably small.

The oxidation of the organic matter in the soil of the gauges would operate in the same way. This process is continually going on, it results in the formation of empty spaces into which air can pass and from which water vapour can diffuse. This change, like the other, becomes apparent in the shallow gauge before it does in the deeper.

Evaporation and drainage are complementary. If the above hypothesis is well founded, it explains why the drainage through the 40 inch gauge is on an average more than through the 60 inch or the 20 inch; it also explains why the 20 inch gauge at first yielded more, and afterwards less, drainage than the 60 inch, and why the 40 inch is starting to behave in the same way. On this view the present agreement between the 20 and 60 inch gauges is accidental and temporary, whilst the difference shown for so many years between the 40 and 60 inch gauges is no abnormal feature, but a normal one. Of the three

gauges, in fact, the 20 inch is the abnormal one and not (or not until recently) the 40 inch.

Two or three results follow from the above change if it has taken place. The differences in the amount of evaporation from the 20 and 60 inch gauges should be accentuated in the dry months, since air penetrates more readily into, and water vapour diffuses out from, the former than the latter. On the other hand the differences should be less marked during wet months. The drainage from the 20 inch gauge should, therefore, in months of low rainfall be less, and in months of high rainfall more, than that from the 60 inch,—more, because the soil is more permeable, and only its greater evaporative power ever makes the drainage less. The data given on page 382 of Dr Miller's paper completely accord with this expectation.

TABLE 3.

Months of low rainfall, average of 35 years.

	Rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
January	2·29	1·79	1·92
February	1·94	1·39	1·44
March	1·88	·92	1·00
April	1·90	·50	·53
May	2·08	·47	·49
Total	10·09	5·07	5·38

In each case the drainage from the 20 inch is less than that from the 60 inch gauge.

Months of high rainfall, average of 35 years.

	Rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
June	2·41	·65	·64
July...............	2·70	·68	·65
August	2·69	·63	·58
September	2·51	·86	·74
October	3·23	1·83	1·64
November	2·82	2·10	2·01
December	2·52	2·02	2·01
Total	18·88	8·77	8·27

Here the drainage from the 20 inch gauge is greater than that from the other.

These figures relate to the whole period. The monthly values for each separate year up to 1880 are given by Lawes, Gilbert, and Warington in the *Journals of the Royal Agricultural Society for* 1881 (Vol. XVII. p. 330). From these it is evident that the relationship brought out in Table 3 above only begins to hold after the first four years, *i.e.* after the changes in the gauges had begun. During these four years, as one would expect, the 20 inch gauge allows more water to percolate even in dry months than the 60 inch.

Months of low rainfall (Jan.–May) 1871–1874.

Mean rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
9·646	3·78	3·48

In subsequent years the order is reversed, as shown in Table 3.

A similar, but less definite, relationship is shown by the nitrification figures. Had there been no alteration in the gauges the 60 inch gauge, having the greatest depth and the greatest amount of nitrifiable organic matter, would be expected to yield the largest quantity of nitrates, the 40 inch would give less, and the 20 inch least. If, however, the changes suggested above have taken place, the 20 inch gauge, being now more open to atmospheric influences than formerly, should yield a larger quantity of nitrates than the 60 inch. On the other hand, the 40 inch gauge, having suffered less change, should yield less.

Bearing in mind Dr Miller's caution on p. 390, that "the relation of nitric nitrogen to the quantity of drainage is somewhat complicated," it is nevertheless evident that the nitrates do vary in the manner just indicated.

TABLE 4.

Nitrogen as nitrates per acre in the drainage for 7 year periods.

	20 inch gauge	40 inch gauge	60 inch gauge
1877–1884	40·55	34·69	39·62
1884–1891	32·53	28·84	29·97
1891–1898	29·72	26·58	29·32
1898–1905	29·48	26·55	28·45

Calculated as percentages on the values for the 60 inch gauge.

1877–1884	102·6	87·56	100
1884–1891	108·5	96·23	100
1891–1898	101·4	90·65	100
1898–1905	103·6	93·90	100

The foregoing evidence all seems to point to a secular change in the drain gauges resulting in an increased evaporation of water. It is suggested that the change is brought about by the diminution of organic matter in the soil of the gauges and the action of rain in washing out the finest particles.

SOME OBSERVATIONS ON THE ASSIMILATION OF ATMOSPHERIC NITROGEN BY A FREE LIVING SOIL ORGANISM.—AZOTOBACTER CHROOCOCCUM OF BEIJERINCK.

By S. F. ASHBY, B.Sc.,

Carnegie Research Fellow, Rothamsted Experiment Station.

Some years before the appearance of Hellriegel and Wilfarth's work on the sources of nitrogen of leguminous plants, and while the part played by atmospheric nitrogen in the nutrition of crops was under active discussion, Berthelot was making some exact observations on the behaviour of uncropped soils towards the free element. He found that when 50 kilograms of air-dry arable soil were exposed to the air and rain in a vessel for seven months a great increase in the nitrogen content could be observed; the total nitrogen of the original soil had increased from 50 grams to 63, or a gain of over 25 per cent. after allowing for the small amount of combined nitrogen brought down by rain; in another experiment where the soil had first been washed free from nitrates, a gain of 46 per cent. of nitrogen was proved. In many other cases, however, the gain was only from 10–15 per cent. of the original nitrogen present in the soil. Gains have also been observed by many other observers but always much lower than those of Berthelot first mentioned. Berthelot was able to show that the gain of nitrogen in a soil kept in the dark was only half as great as in the same soil exposed to light; while, however, he could observe no increase in the nitrogen of his soil during the winter, Koch has found a considerable increase during that time in soil which was kept in a heap and shovelled over from time to time. Berthelot, observing that when the soil had been previously steamed at 100° no gain of nitrogen followed, concluded that soil organisms must be concerned in the fixation; he was unable, however, to separate by plate methods, a single

one which could assimilate free nitrogen in pure culture. In 1894 Winogradsky published his researches on a bacterium which could fix the free nitrogen of the air while growing in pure culture in a solution containing only dextrose and nitrogen-free salts; this organism he called *Clostridium pasteurianum*. It forms spores, is an anaerobe, and belongs to the group of butyric acid bacteria. The sugar is broken into carbonic acid, hydrogen, butyric acid, acetic acid and butyl alcohol, and for every gram of sugar consumed about 2 mgs. of nitrogen are assimilated from the air. In impure culture directly from the soil up to 3 mgs. of nitrogen are fixed for every gram of sugar decomposed.

During the period between Berthelot's work and the discovery of this organism the view had gained ground that algae growing on and near the surface of soil were able to fix nitrogen directly. In 1888 Frank had observed such a growth on sand exposed to light, and had found that the upper layer showed a considerable increase of nitrogen. In 1892 Schloesing and Laurent proved, both by determining the nitrogen fixed by a soil in a closed vessel, and by observing the diminution of the nitrogen gas in the enclosed air, that a soil exposed to light gains in nitrogen if algae are allowed to grow on the surface, and that the gain is confined to the upper few millimetres of depth. They did not however, employ a sterile soil nor pure cultures of algae. Kossowitsch working with pure cultures of two green algae found no fixation, but observed a considerable increase of soil nitrogen by growing them mixed with soil bacteria. Later Krüger and Schneidewind employing pure cultures of many other *Chlorophyceae* could also find no nitrogen fixation. Hellriegel and Déherain had also found very large increase in the nitrogen content of sand in pots when exposed to light, but always accompanied by a development of algae. In the light of such results the conclusion has been drawn that the algae cannot assimilate free nitrogen alone but only in concurrence with soil bacteria, the former producing carbohydrates which are used by the bacteria as a source of energy for the actual nitrogen fixation. Winogradsky's organism showed a very low efficiency of fixation for sugar consumed and was also strictly anaerobic, so that, as fixation in soil had been observed to be most abundant under aerobic conditions and often considerable in amount, the probability seemed strong that other and more active bacteria remained to be discovered. In 1901, Beijerinck was able to report upon a couple of closely allied organisms which gave a very abundant growth under highly aerobic conditions in solutions of carbohydrates containing no combined nitrogen; these he named

7 *Azotobacter chroococcum* and *Azotobacter agilis*. The former was widely distributed in cultivated soils and even in dune sand, the latter was only found in river and canal waters. *A. chroococcum* was obtained from soil by the method of 'elective culture,' so successfully employed by Winogradsky for the isolation of the nitrifying organisms. A few grams of soil were introduced into a solution of the composition:

Tap water	100
Mannite	2
Dipotassium phosphate	·02;

and incubated at a temperature of from 20°–30° either in diffused daylight or in the dark. After a few days a strong film formed on the liquid, consisting mainly of the organism, and by transferring a little to an agar made with the same solution and allowing isolated colonies to develop, a pure culture was obtained.

The mannite can be replaced by dextrose, levulose, cane sugar, dextrin, propionates, butyrates, and acetates, all of which are oxidised, according to Beijerinck, to carbonic acid and water. In mannite solution the growth is both active and fairly pure, whereas in dextrose more foreign organisms develop and cause acidity; and in propionates, butyrates, &c., the growth is very pure but less rapid and abundant. Beijerinck secured a fixation of nitrogen up to 7 mgs. for every gram of mannite or dextrose oxidised in the cultures directly seeded with soil, and similar amounts by inoculating repeatedly with the impure film. In pure culture the *Azotobacter* refused to grow and fix nitrogen in two cases out of four and in the other two fixed 2·70 and 5·50 mgs. N per litre of culture, or only ·13 and ·27 mgs. N for 1 gram of carbohydrate. By combining pure cultures of it with spore-forming butyric organisms (*Granulobacter* sp.) or with non-spore formers separated from the films (*Radiobacter* and *Aerobacter*), he obtained better fixation, up to 5·9 mgs. N per gram sugar consumed. He made no attempt to get *Granulobacter* species alone to fix nitrogen and his attempts with *Radiobacter* and *Aerobacter* were also unsuccessful; nevertheless he asserts that all *Granulobacter* sp. can fix nitrogen and that *Azotobacter* cannot do so alone (although the above-mentioned results disprove the statement). Beijerinck supposes that in mixed cultures the *Granulobacters*, *Radiobacter* and *Aerobacter* gain the power to fix in the presence of *Azotobacter*, which grows itself at the expense of the combined nitrogen escaping from them into the solution.

Very soon, however, Gerlach and Vogel, Freudenreich and Koch, showed that *A. chroococcum* was able to fix considerable quantities of nitrogen in perfectly pure culture. Gerlach and Vogel found that old cultures of the *Azotobacterium* became weaker in their fixative power, and that the amount of N fixed per gram of sugar consumed depended both upon aeration and the concentration of the sugar; 12 grams per litre was found most favourable, giving a fixation of 9–10 mgs. N per gram of sugar oxidised.

Freudenreich has found about the same ratio for cultures on gypsum, and Koch for those on agar. Gerlach and Vogel have proved that the organism cannot grow without phosphoric acid and calcium, but can do without magnesium and potassium. As regards potassium their results are not conclusive, since while as much N was fixed in 20 days without it as in a culture to which it was added, after 40 days there had been no further fixation in the former solution, but it had nearly doubled in the latter, suggesting that the traces of potash in the chemicals and dissolved from the glass during sterilisation had been enough to carry on development for a time only.

The Author's Observations.

In order to gain the first or impure culture of *Azotobacter* from soil a solution of the following composition has been used:

Mannite	12 or 20 g.
Monopotassium phosphate ...	·2 g.
Magnesium sulphate cryst. ...	·2 g.
Sodium chloride	·2 g.
Calcium sulphate	·1 g.
Distilled water	1 litre.

The phosphate was dissolved separately and rendered just alkaline to phenol-phthalein by the addition of N/10 sodium hydrate. 75 c.c. or 100 c.c. of the solution was then put into Erlenmeyer flasks of 250 or 300 c.c. capacity and sterilised in an autoclave at 130° for 15 minutes. Each flask then received ·5 g. of calcium carbonate (precipitated) and 1 g. of soil. Incubation was maintained at about 30°. After two days the solution becomes somewhat clouded and gas bubbles rise from the bottom of the flask; on the third day a gelatinous colourless ring forms on the glass along the edge of the liquid and from it a film rapidly extends and covers the whole surface of the liquid; this is at first white, opaque, dry, often very reticulate, and it may or may not be filled with gas bubbles. After a couple of days

it becomes yellow, later brown and finally almost black. The last state is arrived at in from 7–10 days, and the liquid beneath is usually quite clear. By the tenth day the mannite has usually disappeared, sometimes even at the end of a week. If allowed to remain after the disappearance of the carbohydrate the solution begins to cloud again and the film breaks up and falls to the floor of the flask; at the same time a marked putrefactive odour is perceived, due to the attack on the proteid of dead *Azotobacter* cells by putrefactive bacteria. If it is proposed to determine the amount of nitrogen fixed this latter stage must not be allowed to proceed since it results in loss of nitrogen as ammonia and other volatile compounds. The nitrogen has been determined by transferring the whole contents of the flasks to Jena glass combustion flasks, acidulating with sulphuric acid and evaporating to a small volume before adding the concentrated acid. As a rule there is an odour of butyric acid and fruit ether from the culture, the acid odour being most marked during concentration. When the butyric odour is much in evidence *Clostridia* can be found in flocks overlying the carbonate and soil on the bottom of the flask but rarely in the surface film, and this is accompanied by a relatively poor fixation of nitrogen. This occurs more often in cultures to which no calcium carbonate has been added. To obtain pure cultures a little young film is well shaken in sterilised distilled water and a drop spread over mannite agar in a petri dish. The agar must first be well washed for some days with frequent changes of cold water after cutting up, then partly dried with the aid of the filter pump, and finally dissolved in the culture solution mentioned above in the proportion of 1½–2 g. per litre. If unwashed agar is used too many colonies of foreign organisms develop before the *Azotobacter* and fuse with its colonies. Even in purified mannite agar transparent watery colonies of fluorescent bacteria appear in one day, and the *Azotobacter* colonies which appear about a day later often grow over them or fuse with them so that one cannot depend on getting a pure culture by inoculating off the first plate. In a second plating one can generally find colonies of *Azotobacter* isolated enough to secure a pure culture after transferring to an agar slant.

Appearance of Azotobacter chroococcum.

In the young impure films on solutions inoculated with Rothamsted soil the organism appears at first mainly in oval forms, often united in pairs, but also as cocci and diplococci, the former measuring 4–5 μ in length by 3 μ in width and the latter 2·5–4 μ in diameter for the

individual cell. If stained with iodine in potassium iodide many only assume a golden yellow colour, others are more brown and many of the cocci are of a deep red-brown. This latter colouration has been shown by Heinze to be due to glycogen similar to that which occurs in yeast cells. In old cultures the cells are practically all stained red-brown by iodine. If a little of a very young film is examined in a hanging drop of culture liquid, a cell here and there will be observed to suddenly become sluggishly motile and slowly cross the field of vision. Zettnow has found that the motion is due to a bundle of cilia at one pole. On mannite agar the colonies first appear as milky white glistening drops, round and convex, which under a low magnification show a coarsely granular structure extending to the margin. The colonies rapidly increase in size and after a week or more become brown at the centre, with concentric rings alternating dark and white to the circumference and darker streaks radiating from the centre outwards. In old cultures, where the agar has partly dried up, the cells are often united in Sarcina-like packets, the cell walls are much swollen, and the contents are aggregated to a small ball at the centre; at the same time giant cells both round and elongated can be seen filled with oil drops, and often a number of involution forms drawn out into long threads with false septa.

Some cultures after repeated transference on agar slants lose the power of turning dark with age and do not again recover it. On peptone beef extract agar the organism gives hardly any growth, but grows well when 1 per cent. dextrose is added, though the power of forming glycogen seems to be lost on this medium. In broth containing no added sugar there is practically no growth, the medium remaining clear with the formation of a slight sediment; if dextrose is added there is abundant multiplication at the surface of the liquid and a considerable sediment. To test the purity of cultures, seeding has been made into broth, and plates have been prepared with peptone beef extract agar with dextrose added; if the culture was pure the only colonies appearing on the peptone dextrose medium were those of *Azotobacter*.

Fixation of nitrogen by pure cultures.

In some early experiments with pure cultures of the Rothamsted organism great difficulty was found in getting it to grow in solutions containing either mannite or dextrose and prepared either with tap water or distilled water. After five weeks there was very slight

multiplication, and the quantity of nitrogen fixed was infinitesimal, although the cultures used had been freshly isolated, and were growing vigorously on the solid medium. Later, in order to secure an abundant inoculation the following method was adopted: 50 c.c. portions of dextrose and mannite culture solutions containing 12 g. to the litre and made up with tap water, received ·5 g. calcium carbonate, and were then sterilised. Flasks of 250 c.c. capacity received 10 c.c. of dextrose or mannite agar, and were then sterilised. The culture of *Azotobacter* was rubbed over the surface of this agar and allowed to develop for two or three days at 30°. When the growth had become abundant 50 c.c. of a sterile liquid medium, made up with tap water containing 12 g. of either mannite or dextrose in the litre and 1 per cent. calcium carbonate, was poured on to it and the flasks incubated at 30°. As a result of this method of inoculation the organism multiplied abundantly and formed a film on the surface of the liquid. Controls prepared in the same way but not inoculated were incubated with the cultures.

The results, with these pure cultures of organisms from three different places are given in the following table:

TABLE I.

Nitrogen fixed in milligrams for 1 gram of carbohydrate.

Origin	Carbon source	Time	1st plating	2nd plating	3rd plating
		days	mgs.	mgs.	mgs.
Mombasa, E. Africa	mannite	20	7·30	5·12	7·24, 7·82
	glucose	20	6·73	6·18	7·85
Cairo, Egypt	mannite	20	7·64	—	—
	glucose	20	5·73	—	—
Rothamsted	mannite	40	4·91	3·77	—
	glucose	40	4·62	3·57	—

A further reference will be made to the organisms from Africa under the final heading.

To test whether the mannite had disappeared, a drop or two of the solution was evaporated to dryness on a watch-glass and examined under the microscope for the characteristic needles; for dextrose by running a little of the culture into a boiling solution of methylene blue in caustic potash and observing whether the colour was discharged. Both tests were employed by Beijerinck.

Fixation was considerable in all cases and with the Mombasa *Azotobacter* was not weakened after three successive platings. The Rothamsted organism developed much more slowly, and gave a lower yield of nitrogen, thus showing a marked diminution in power to fix after the second plating.

Fixation of nitrogen by impure cultures.

Soil from many of the Rothamsted plots has been tested for the presence of *Azotobacter*, and the amount of nitrogen fixed in solutions seeded with it has been determined.

The following experiment brings out the influence of aeration on nitrogen fixation. The solution used contained 20 g. of mannite in the litre. The cultures were incubated at 30° for ten days, and each received 1 g. of soil as inoculation, and ·5 g. $CaCO_3$.

Soil	Solution	Total N fixed	N fixed per 1 g. mannite
Barnfield		mgs.	mgs.
1 c.	100 c.c.	11·9	5·95
,,	50 c.c.	9·2	9·20
4 c.	100 c.c.	9·1	4·55
,,	50 c.c.	8·8	8·80

The flasks used were of 250 c.c. capacity in which 50 c.c. of solution only forms a thin and fully aerated layer.

It is evident that the superior aeration with only 50 c.c. of solution caused a much greater fixation of nitrogen per gram of mannite oxidised. A stronger film of *Azotobacter* also developed.

In the following table will be seen the amounts of nitrogen fixed both where *Azotobacter* developed and where no trace of it could be found.

Table II.

50 c.c. solution, 2 g. soil and incubated 30 days at 25°–30°.

Soil	N fixed for 1 g. mannite	*Azotobacter*
	mgs.	
Broadbalk Wilderness + $CaCO_3$	8·80	abundant
,, ,, ,, alone......	7·30	,,
Soil from drain gauge + $CaCO_3$	6·60	fairly abundant
,, ,, ,, alone......	5·10	,,
Geescroft Wilderness + $CaCO_3$	3·53	absent
,, ,, ,, alone	2·60	,,
Harpenden Common + $CaCO_3$	3·73	,,
,, ,, ,, alone	2·90	,,
Park soils		
Plot 1, unlimed........	3·94 and 1·15	,,
,, limed.......................	3·66 and 3·80	,,
Plot 4·2 unlimed	3·59	,,
,, limed	3·35	,,
Plot 9 unlimed	3·21	,,
Average fixation	6·95	*Azotobacter* present
,, ,, ...	3·22	,, absent

In presence of *Azotobacter* the average yield of nitrogen was more than doubled. When *Azotobacter* was absent there was a long continued production of gas and a foamy very thin film, a strong odour of butyric acid and flocks of *Clostridia* on the bottom of the flasks. It is evident then that fixation of nitrogen takes place even in the absence of *Azotobacter*, but is always very low in amount, approaching the yield found by Winogradsky for his *Clostridium*. It may be added that quite recently Thiele has obtained from soil several *Clostridia*, distinct from *C. pasteurianum* and capable of fixing up to 3 mgs. of nitrogen for every gram of dextrose fermented. Some of them also ferment mannite.

In all experiments with Rothamsted arable soils where fixation of nitrogen occurred *Azotobacter* was abundant in the film on the cultures.

The following are the best results obtained with pure and crude cultures of the organism.

Pure cultures	Mixed soil cultures
mgs. N fixed for 1 g. mannite oxidised	mgs. N fixed for 1 g. mannite oxidised
7·30	9·2
7·64	8·8
7·24	8·8
7·82	9·2
Average 7·50	9·22
	9·53
	Average 9·12

It seems that in some way concurrence with other bacteria in the primary cultures acts favourably on fixation, provided the reaction remains neutral or alkaline; free acid (as in cultures without calcium carbonate which give a butyric fermentation) seriously affects the growth of *Azotobacter*.

Conditions favourable for fixation of Nitrogen.

1. *Aeration.*

The value of an abundant supply of air is well shown in the results of Table I.; in this case a solution of only half the depth with a similar extent of surface gave nearly twice as much fixation per gram of mannite oxidised. The more rapid growth on solid media as against liquids is also a proof of the great requirements of the organism for oxygen. In shallow cultures the injurious butyric fermentation, which is more or less anaerobic, is partly or wholly suppressed.

2. *Presence of a base.*

In Table II. the amount of fixation in the presence and absence of calcium carbonate is shown. It will be seen that where *Azotobacter* was present the addition of base increased fixation. In the following experiment the amount of nitrogen gained was determined at the end of ten days in 100 c.c. solutions containing 2 g. of mannite, with and without the addition of ·5 g. calcium carbonate. The solutions were inoculated with 1 g. of soil and incubation was conducted at 29°–30°.

Soil	Depth	$CaCO_3$ added	No addition of $CaCO_3$
		Nitrogen fixed mgs.	Nitrogen fixed mgs.
Little Hoos	10 cms.	18·13	5·33
,, ,, ...	20 cms.	9·78	4·79
,, ,, ...	30 cms.	5·21	3·98
Agdell, unmanured ...	10 cms.	9·86	4·61
,, full manured...	10 cms.	5·86	0·00

The figures show the total amount of fixation, the mannite not having been all oxidised in most cases. The *Azotobacter* has developed more rapidly in the series with calcium carbonate, which has secured growth and fixation in all cases, whereas both were wanting in one of the solutions receiving no carbonate.

In the following experiment an attempt was made to judge of the distribution of *Azotobacter* in several plots on Agdell Field at Rothamsted, where a four course rotation has been maintained with and without manures for over 50 years. One half of the plots had been left fallow every fourth year, and the other half seeded with beans or clover. The manures, all applied to the root crop, were on one-third of the land, phosphates and potash only; on another third, the same mineral manures and nitrogen; and on the remaining third, no manure. At some time before the experiments began the no manure, and about half the mineral plots, had been heavily chalked in the past, but the other half of the mineral plots, and the minerals with nitrogen plots, were unlimed, containing at present only a trace of carbonate. The soil was taken at a depth of 15 cms., and 1 g. was seeded into 75 c.c. solutions containing 12 g. mannite to the litre. To some solutions ·5 g. calcium carbonate was added, to others the same amount of magnesium carbonate, but most were left neutral. The flasks were incubated at 30° degrees for twelve days. Early in May, 1906, when the samples were taken, one half of each plot was under alsike clover, and the other half in fallow.

Table III.

Plot	N fixed for 1 g. mannite			Film appeared after		
	Neutral	With $CaCO_3$	With $MgCO_3$	days	days	days
	mgs.	mgs.	mgs.	Neutral	$CaCO_3$	$MgCO_3$
Minerals-fallow,						
chalked end	6·50	—	—	3½	—	—
unchalked end	4·75	6·80	8·00	4	4	6
Minerals-clover,						
chalked	5·80	—	—	5–6	—	—
unchalked	0·00	4·80	9·22	none	5	6
Unmanured-fallow,						
chalked	6·90	—	—	5–6	—	—
Unmanured-clover,						
chalked	4·30	—	—	7	—	—
Full manured-fallow,						
unchalked	4·20 & 0·00	5·80	9·53	4 & none	5	8
Full manured-clover						
unchalked	0·00	3·78	0·00	none	8	none

All the cultures were made in duplicate, and the figures represent the mean fixation for the pairs. The figures for the neutral solutions show that *Azotobacter* developed in every case from the fallow soils whether containing carbonate of lime or not; two of the clover soil inoculations failed to show any growth of *Azotobacter* and were not analysed, the average fixation from the fallow being 5·06, and from the clover land only 2·52. The chalked parts always gave *Azotobacter*, but there were three failures from the unchalked land, the average fixations being 5·87 and 1·71 respectively. Where there was failure to develop *Azotobacter* in the neutral solution, addition of calcium carbonate secured a film in each case. From the results with the neutral solutions it may be concluded that *Azotobacter* is more abundant in fallow than under clover, and much more abundant in soils well provided with carbonate of lime than in others where the latter is almost absent. The results shown in Table II. present a striking confirmation of the latter conclusion. In the three cases where *Azotobacter* developed and nitrogen fixation was large the soils contained abundance of calcium carbonate. In every case where *Azotobacter* failed to appear, the soils contained only a trace of carbonate, and even addition of carbonate to the solutions was ineffectual. All the latter soils were acid. to litmus.

Magnesium carbonate as base.

As shown in Table III., an addition of magnesium carbonate in place of calcium carbonate caused a very large fixation of nitrogen in three cultures out of four, but the film developed much later. The average fixation with magnesium carbonate was 8·92 mgs., with calcium carbonate 5·80 mgs. The film developed with magnesium carbonate in 6·6 days and with calcium carbonate in 4·6 days. With magnesium carbonate there was 50 per cent. more nitrogen fixed, and a delay of two days in development. Examination of the cultures showed that the film with magnesium carbonate contained far less foreign organisms, and that whereas with calcium carbonate there was a butyric or fruit ether odour, the cultures with magnesium carbonate were quite odourless, even during concentration with acid.

A special experiment was therefore made to compare the influence of the carbonates of magnesium and calcium upon fixation and growth.

To three parallel solutions of 75 c.c. containing 12 g. mannite to the litre, ·5 g. calcium carbonate was given, to another set ·5 g. magnesium carbonate, to a third ·25 g. of each carbonate, and another was left neutral. All were inoculated with ·5 g. soil and incubated for 14 days

at 29°–30°. The amount of nitrogen fixed is expressed as mgs. per gram mannite.

	Neutrals	$CaCO_3$ series	$MgCO_3$ series	$CaCO_3 + MgCO_3$
1.	5·75	7·8	8·12	8·71
2.	4·98	7·15	7·62	7·08
3.	—	6·70	8·25	—
Average	5·36	7·22	8·00	7·89
Film appeared in days	4–5	3–4	8–10	6

During concentration the neutral cultures developed a strongly acid odour, those with calcium carbonate a weaker one, and those with magnesium carbonate, alone or mixed with calcium carbonate, gave no odour. When magnesium carbonate was present, development was greatly delayed, but the yield of nitrogen was again larger, though not to so marked an extent as in the earlier experiment. In pure culture *Azotobacter* gives rise to no acidity, either in solutions or on agar. One must conclude, therefore, that magnesium carbonate not only neutralises more effectually than calcium carbonate any trace of acidity due to foreign organisms in the early stages of culture, but also prevents butyric fermentation, but at first it inhibits the growth of *Azotobacter* itself.

Nitrogen fixation by soils taken at different depths.

Only one experiment has been made in this connexion. The writer has described in another place a method for obtaining and preparing an average soil sample[1]. The soil was taken from a fallow plot at depths of 10, 20 and 30 cms., and equal quantities were seeded into 100 c.c. portions of a culture solution containing 20 g. mannite in the litre. ·5 g. calcium carbonate was added, and incubation maintained at 29°–30°.

Little Hoos fallow soil.

Depth	7 days	10 days	13 days
	mgs.	mgs.	mgs.
10 cms.	6·84	13·13	12·15
20 cms.	3·77	9·78	11·03
30 cms.	2·93	5·21	10·20

[1] "The comparative nitrifying power of soils," *Trans. Chem. Soc.* 1904, vol. LXXXV., p. 1158.

The figures show the total nitrogen gained by the cultures during the times stated. The greatest contrast was after ten days. During this time the mannite had all been oxidised in the cultures inoculated with soil at 10 cms. depth, so that no further gain of nitrogen took place during the remaining three days. With soil from 20 cms. depth most of the sugar had gone after ten days, but with that from 30 cms. not till after thirteen days. The results show that *Azotobacter* is most abundantly present in soil near the surface, and falls off in amount with increasing depth. It is also evident that fixation does not occur uniformly over the period of active oxidation, but increases rapidly up to a point, and then becomes slower, corresponding with the greatly lessened concentration of carbohydrate.

Azotobacter from African soils.

It had been observed that, when a little of a soil which gave a growth of the organism in solutions was rubbed over a mannite agar plate, the characteristic colonies develop in two or three days, together with many other forms which, however, soon cease to grow, while the *Azotobacter* colonies spread rapidly and soon begin to darken.

Mr Hall took with him to South Africa in 1905 a number of such plates, and inoculated them with fresh soil from several parts of the "high veldt," and one with a tropical cultivated soil at Mombasa, in E. Africa. On examination it was found that *Azotobacter* had developed in only two cases, giving a gelatinous nearly black growth, which covered the greater part of the plates. An attempt was made to get the organism in pure culture by growing in nutritive solutions. The material was very impure and contained many amoebae and infusoria, which live largely on the cells of *Azotobacter*. The Mombasa organism was obtained pure by plate inoculations from a film which had slowly formed on a solution of sodium butyrate. The other organism, from a "vlei" soil near Lichtenburg in the Transvaal, did not give even a relatively pure growth, and could not be isolated. The film of the Mombasa organism on the butyrate was white, dry and brittle, and was remarkable for the very large size of the round cells, 5–6 μ diameter, which were rarely united. In pure culture, this Mombasa organism differs from all others examined by the production of a soluble pigment, at first greenish-blue, and later, light yellow in tint, which not only colours the growth on agar, but diffuses into both the solid and liquid media. From the first, the pure culture had no power of turning brown with age. On mannite and

glucose agar the growth is very rapid and more fluid than that given by Rothamsted organisms, and under the microscope the cells are isolated, very small, and almost invariably round; the diameter varying from 2–2½ μ. Iodine in potassium iodide only stains it yellow, so that the power of forming glycogen seems to have been lost. The formation of the pigment is markedly favoured by the presence of calcium carbonate both in solid and liquid media. On one mannite agar plate, where carbonate had been first added but had accumulated at one end, the growth above the carbonate alone produced pigment, which was quite absent on the portion where carbonate was wanting. Calcium carbonate seems to have a specific action on the production of pigment, the colour of which is at once discharged by mineral acids.

The very active fixation of nitrogen by pure cultures of the Mombasa organism even after repeated transference, has already been referred to (see Table I.). Its behaviour towards media containing organic nitrogen is similar to that described for the Rothamsted organism. In a solution containing dextrose and ·02 per cent. nitrogen as calcium nitrate growth is abundant, and the nitrate is very slowly and only partly converted to ammonia.

The Cairo organism was isolated from a sample of fresh soil collected at Ghezirah by Mr R. Aladjem of the Agricultural Experiment Station there. This soil showed a markedly alkaline reaction to litmus paper, due to alkaline carbonate, but the bulk of the carbonate present was calcium carbonate. The crude cultures inoculated with soil were remarkable for the speed with which the film developed and the mannite disappeared, fixation being complete in five days, by which time the film was quite black. The fixation was also identical in amount from cultures with varying amounts of solution, and with and without added carbonate. Even after ten days there was no putrefactive odour, and no trace of a butyric fermentation could be detected. The fixation was in every case 9·2 mgs. nitrogen for 1 gram mannite oxidised.

In pure culture this organism is in every way similar to that from Rothamsted, with the single exception that fixation is with it more rapid and greater (see Table I.) in amount. The power of turning brown, and finally black with age, has been preserved in the pure cultures.

Although the vlei organism from S. Africa was not isolated, it produced a pigment quite similar to the Mombasa form in impure culture, and must certainly be classed with it as one variety. As descriptions by continental authors agree fully with the Rothamsted and Cairo type, one must conclude that there is one variety common to

Europe and extending to Egypt, and another quite distinct found in East and South Africa.

The organism separated by Beijerinck from waters and named *A. agilis*, is a larger form which is very actively motile in young liquid cultures, and also forms a diffusible pigment, green in solutions of salts of organic acids and reddish-violet in the presence of carbohydrates.

Effect of desiccation on Azotobacter chroococcum.

No spores are formed, yet the organism can resist drying up in the air for a long time. A soil, which was known to contain the organism in the fresh condition, after being kept air-dry in bottle for a year, still yielded an abundant growth after inoculation into a mannite solution. Old cultures of the organism on agar which had dried down to a leathery consistency, after many months still showed abundant growth after pouring a fresh culture solution over them. It is evident then, that the organism can be freely distributed in dust by the wind.

General Remarks.

Several observers have been struck by the resemblance of the organism to some of the unicellular Algae. Beijerinck believes it to be closely related to Winogradsky's *Chromatium*, while Benecke and Keutner consider it a colourless form of one of the *Cyanophyceae* namely *Aphanocapsa*, but no one has yet been able to induce it to produce chlorophyll by cultivation in light. Attempts have been made to bring about nitrogen fixation by seeding pure cultures into sterilised and unsterilised soil, but as yet without success. Similar experiments are now in progress at Rothamsted. A. D. Hall has recently reported on two cases of considerable nitrogen increase in Rothamsted soils allowed to run wild for many years. In one, showing the greater increase, *Azotobacter* was abundantly present, in the other it could not be found, but butyric organisms were present (see Table II.).

Some references to the more important papers dealing with the subject are given below. Where the *Centralblatt für Bakteriologie* is cited the Second Part is always meant.

BEIJERINCK, M. W. Über oligonitrophile Mikroben. *Centr. f. Bakt.* **7**, 561. (Original communication in *Archives Néerlandaises*, II. **8**, p. 190.)

BEIJERINCK, M. W. and A. VAN DELDEN. Über die Assimilation des freien Stickstoffes durch Bakterien. *Centr. f. Bakt.* **8**, p. 3. (Original in *Archives Néerl.* II. **8**, p. 319.)

Berthelot, M. *Comptes rend.* **101** (1885), **102** (1886), **104** (1887). An Epitome of all results in "Chimie végétale et agricole," 1899.

Frank, B. *Landw. Jahrbücher*, 1888, p. 421.

Freudenreich, Ed. von. *Centr. f. Bakt.* **9**, p. 514.

Gerlach u. Vogel. *Centr. f. Bakt.* **8**, p. 669; **9**, p. 817; **10**, p. 636.

Hall, A. D. On the Increase of Nitrogen in land allowed to run wild. *Journ. Agric. Science*, Vol. **1**, p. 241.

Keutner. Extract. *Centr. f. Bakt.* **13**, p. 554.

Koch, A. Lecture before Economic Society of Saxony, Dec. 1903. Article in Lafar's *Handbuch der technischen Mykologie*, 2nd Ed., 1904.

Kossowitsch, P. *Bot. Ztg.* **52**, p. 97, 1894.

Krüger u. Schneidewind. *Landw. Jahrb.* **29**, p. 771, 1900.

Schloesing et Laurent. *Comptes rend.* **113**, 1891 ; **115**, 1892.

Winogradsky, M. S. Recherches sur l'assimilation de l'azote libre de l'atmosphère par les microbes. *Archives des Sciences biologiques* (St Petersburg), 1894, **3**, p. 297. Clostridium pasteurianum, seine Morphologie und seine Eigenschaften als Buttersäureferment. *Centr. f. Bakt.* **9**, p. 43.

SOME OBSERVATIONS ON "NITRIFICATION."

By S. F. ASHBY, B.Sc.,

Carnegie Research Fellow, Rothamsted Experiment Station.

It is now a well-established fact that the ammonia, which is formed in the cultivated soil by the breaking down of nitrogenous organic matter through the action of bacteria and moulds, is itself converted by oxidation into nitrates. The latter process, "nitrification" proper, is effected in two stages by two distinct species of bacteria, the one carrying the oxidation to nitrite, and the other changing the latter compound into nitrate. It is also known that, in the absence of a base, nitrification does not take place. The most usual and most available soil base is calcium carbonate, which is, however, present in some arable soils to only a very small amount, less than ·05 per cent.; in such soils, and in others where its presence cannot be detected, nitrification is still active, a fact which seems to suggest that other substances may replace it and serve as bases for nitrification.

In order to test the latter point an attempt has been made to set up nitrification in a solution containing an ammonium salt the necessary base being supplied by such commonly occurring soil-constituents as kaolin, modelling clay, and ferric hydrate, the latter both in the freshly precipitated form and as "iron rust." The solution had always shown itself very favourable for nitrification in the presence of calcium carbonate; the composition was as follows:

Ammonium sulphate	·5 gram.
Sodium chloride	·5 "
Cryst. magnesium sulphate	·15 "
Cryst. ferrous sulphate	·10 "
Monopotassium phosphate	·25 "
Distilled water	1 litre.

In a first experiment 100 c.c. portions were sterilised in Erlenmeyer flasks of 250 c.c. capacity, and one gram of either kaolin, modelling clay or freshly precipitated ferric hydrate was added, together with ·2 gram of a soil possessing active nitrifying power in order to convey the necessary organisms. After sterilisation the solution had an acidity equal to 2·3 c.c. N/10 alkali, with phenol-phthalein as indicator. For each substance there was a pair of acid and a pair of neutral solutions. Incubation lasted for 49 days at 29°–30° C. At the end of that time the cultures were examined for ammonia, nitrite and nitrate. In both the acid and neutral solutions containing kaolin and modelling clay the ammonia reaction was strong, that for nitrite absent and the nitrate reaction very faint; with ferric hydrate there was still ammonia but also a strong nitrite reaction. A pair of control solutions to which ·2 calcium carbonate had been added nitrified completely in 20 days. The total absence of nitrification in the flasks containing kaolin and modelling clay after seven weeks (the trace of nitrate was from the air) indicates that those substances cannot act as bases for nitrification. To try and confirm the result for ferric hydrate, a further series of cultures were made both with the freshly precipitated form and with finely ground "iron rust." The quantitative results are set out in Table I.

TABLE I.

Base	Period of incubation	Reaction	N as Ammonia	N as Nitrite	N as Nitrate	Total N	Parts p.c. nitrified	Excess of nitrified N due to base
1st Set	days		mgs.	mgs.	mgs.	mgs.		
Ferric hydrate 1.	49	acid	7·13	2·95	none	10·08	29·3	
,, ,, 2.	,,	,,	7·35	2·45	,,	9·80	24·9	
,, ,, 1.	,,	neutral	6·44	3·20	,,	9·64	33·2	
,, ,, 2.	,,	,,	6·30	3·45	,,	9·75	35·4	
2nd Set								
Ferric hydrate 1.	32	acid	7·42	none	2·80	10·22	27·4	20·2
,, ,, 2.	32	,,	7·39	,,	2·70	10·09	26·7	19·5
,, ,, 3.	43	,,	7·21	,,	3·30	10·51	31·4	24·2
,, ,, 1.	43	neutral	5·60	4·34	none	9·94	43·6	17·9
,, ,, 2.	43	,,	5·81	4·10	,,	9·91	41·4	15·7
Iron rust 1.	32	acid	6·30	none	4·06	10·36	39·2	32·0
,, ,, 2.	32	,,	6·30	,,	4·06	10·36	39·2	32·0
,, ,, 3.	43	,,	6·23	,,	4·13	10·36	39·9	32·7
,, ,, 1.	43	neutral	4·62	,,	5·67	10·29	55·1	29·4
,, ,, 2.	43	,,	4·20	,,	5·98	10·18	58·7	33·0
Soil alone	43	acid	9·94	,,	0·77	10·71	7·2	
Soil alone 1.	43	neutral	7·77	,,	2·80	10·57	26·5	
,, ,, 2.	43	,,	7·77	,,	2·60	10·43	24·9	

Excluding the first set, in which no controls with soil alone were made, it will be observed that, after allowing for the nitrification in the controls of the second series, the ferric hydrate has accounted for the oxidation of about 20 per cent. of the ammoniacal nitrogen and the "iron rust" for over 30 per cent., and that there is practically no increase after 32 days. Nitrification has therefore stopped at a certain point in spite of the great excess of base. This result is most probably due to the hydrolysis of the ferric nitrite or nitrate formed, the acid being set free and preventing further action when it had attained a certain concentration. Soil conditions would be more favourable for the continuation of nitrification, especially below vegetation actively withdrawing the nitric radical. One may conclude therefore that ferric hydrate may act as a soil base inferior to calcium carbonate.

NITRIFICATION OF AMMONIA ABSORBED BY CLAY AND PEAT.

It is well known that both clay and peat absorb the ammonia of an ammonium salt, the action being a substitution whereby the ammonia displaces a base from its combination with hydrated aluminium silicate in the one case and with humic acid in the other; the base comes into solution combined with the acid of the ammonium salt, so that no change in the reaction of the medium can be observed. With regard to clay this only holds true if the solution of the neutral salt is weak, as Veitch has shown that 5 per cent. solutions of neutral salts of the alkalis give rise to aluminium and ferric salts which hydrolyse and produce an acid solution.

It was thought of interest to observe whether the double ammonium salts formed with peat and clay were susceptible of nitrification in the absence of any specially added base. It may be added that these combinations do not yield any ammonia to pure water.

For the experiment modelling clay and a close black homogeneous peat taken from some depth were used. 100 grams of modelling clay were shaken for twelve hours with 3 litres of N/50 ammonium chloride solution, and 100 grams of peat were similarly treated with 2 litres of the same solution. 5 grams of the washed materials were added to 100 c.c. portions of a nitrogen-free mineral solution, together with ·2 gram of a soil having active nitrifying power. Control solutions contained ·1 gram calcium carbonate in addition to either clay or peat. The medium had the following composition:

Cryst. magnesium sulphate	50 mgs.
Sodium chloride	50 „
Monopotassium phosphate	50 „
Distilled water	1 litre.

The solutions were either left acid or made neutral to phenolphthalein. Incubation lasted 60 days at 30° C. The quantitative results are given in Table II.:

TABLE II.

Substance	Reaction	N as Ammonia	N as Nitrate	Total Nitrogen
Modelling clay ....1.	acid	0·42	1·96	2·38
„ „ ....2.	„	0·28	1·96	2·24
„ „ ....1.	neutral	0·84	—	—
„ „ ....2.	„	0·56	2·10	2·66
„ „ + ·1 g. $CaCO_3$ 1.	„	0·14	2·66	2·80
„ „ „ 2.	„	0·21	2·24	2·65
Peat ....1.	acid	1·68	0·56	2·24
„ ....2.	„	1·82	0·28	2·10
„ ....1.	neutral	1·82	0·42	2·24
„ ....2.	„	1·82	0·42	2·24
Peat + ·1 g. $CaCO_3$ ....1.	„	0·14	2·10	2·34
„ „ ....2.	„	0·00	2·24	2·24

No nitrite was found in any case. It will be observed that both with the clay and the peat an addition of calcium carbonate has caused a practically complete nitrification of the absorbed ammonia. The modelling clay cultures without base show also a nearly complete nitrification, but this is by no means the case with the peat, the small amount of nitrate found in the absence of base having come from the air. The ammonia absorbed by peat appears not to be nitrifiable in the absence of base, but is so when with clay. The conclusion would have been, however, more convincing with larger quantities of material.

IS A NEUTRAL AMMONIUM SALT DIRECTLY NITRIFIABLE?

In agricultural text-books the statement is often found that nitrification does not progress except in the presence of a base which neutralises the acid formed. The implication is that at the outset some nitrous acid is formed which, unless neutralised by a base, inhibits

further oxidation of ammonia by the organism. This occurrence of free acid has, however, never been proved. Winogradsky observed that perfectly neutral solutions containing ammonium sulphate inoculated with pure cultures of the nitrite organism showed neither at first nor after long keeping any trace of nitrification. Warington has also shown that a dilute ammonium carbonate solution (or a diluted stale urine) seeded with the organism nitrifies to the extent of exactly half the ammoniacal nitrogen, ammonium nitrite being produced, or, in the presence of both organisms, ammonium nitrate. Neither the sulphate nor the nitrate of ammonium are capable therefore of any degree of nitrification in the absence of a base. Both observers have concluded that ammonium carbonate is alone nitrifiable, but only completely so if another carbonate is present to decompose the nitrate of ammonium produced and form fresh ammonium carbonate. In the course of work upon the comparative nitrifying power of soils the writer has observed that control culture solutions to which insoluble carbonates have been added, but no organisms, show after some weeks at constant temperature a marked loss of ammoniacal nitrogen. This loss is due to a reaction between the ammonium sulphate in solution and the added carbonate, ammonium carbonate and a sulphate being formed, the former gradually volatilising from the solution. The amount of loss of ammonia depends upon the rate of formation of ammonium carbonate, which, other conditions remaining constant, depends upon the amount and the kind of carbonate added. The solution from which these losses have been observed had the following composition:

Ammonium sulphate	·5 gram.
Sodium chloride	·5 „
Monopotassium phosphate	·25 „
Cryst. magnesium sulphate	·15 „
Cryst. ferrous sulphate	·10 „
Distilled water	1 litre.

100 c.c. portions were sterilised in Erlenmeyer flasks of 250 c.c. capacity in a steam autoclave at a temperature of 125° for 15 minutes. During sterilisation no loss of ammonia occurred, nor was there any change in the ammonia content after incubation at 30° for two months. During sterilisation the iron was changed to the ferric state and precipitated as phosphate, the solution showed an acidity equal to 2·3 c.c. N/10 alkali with phenol-phthalein as indicator. The solution contained about 10·5 mgs. ammoniacal nitrogen. ·2 gram calcium carbonate, ·17 gram

magnesium carbonate and ·25 gram copper carbonate in a finely precipitated state were added to the unneutralised solutions and incubation maintained at 30° for varying periods; the losses of ammoniacal nitrogen with the different carbonates are shown in the following table:

	$CaCO_3$	$MgCO_3$	$CuCO_3$
Loss in 20 days			
1.	0·87 mgs.	2·3 mgs.	—
2.	0·70 ,,	2·1 ,,	—
3.	0·85 ,,	2·2 ,,	—
4.	0·98 ,,	2·5 ,,	—
Average loss	0·85 (8·02 p. c.)	2·27 (21·41 p.c.)	—
Loss in 30 days			
1.	1·18	—	—
2.	0·98	—	—
3.	1·16	—	—
4.	1·01	—	—
Average loss	1·08 (10·19 p.c.)	—	—
Loss in 56 days	—	—	0·21 mgs. (1·98 p.c.)

It is evident that magnesium carbonate reacts much more rapidly with the ammonium salt in solution than calcium carbonate, causing a loss of ammonium carbonate by volatilisation nearly three times as great in the same time. This property must be connected with the often observed fact that magnesium carbonate sets nitrification going in a solution several days earlier than calcium carbonate. On the other hand, the very insoluble copper carbonate has reacted so feebly with the ammonium salt that even after eight weeks the loss of ammonia is below 2 per cent. A pair of solutions containing ·25 gram copper carbonate and seeded with ·2 gram of a soil having very active nitrifying power failed to show any trace of nitrification after 40 days at 30°, although control cultures with calcium carbonate had completely nitrified in 18 days. That the copper carbonate had exercised no poisonous action on the organisms was proved by the fact that when a little calcium carbonate was added at the end of the 40 days, the ammonia became completely nitrified in less than three weeks. The incapacity of copper carbonate to set nitrification going must therefore be correlated with its very low power of reaction with the ammonium salt.

Comparison of the figures for calcium carbonate after 20 and 30 days shows that the loss of ammonia is greatest during the early period, nearly

79 per cent. of the loss during 30 days having occurred during the first 20 instead of only 67 per cent. had the loss been uniform.

The above observations confirm the conclusions of Winogradsky and Warington, that an ammonium salt is not directly nitrifiable and that the function of the base in nitrification is to secure the formation of ammonium carbonate, which alone is directly nitrifiable. From this standpoint kaolin and modelling clay cannot set up nitrification because they cannot free ammonia from a neutral salt, and ferric hydrate acts positively because it does react with the neutral salt.

Influence of Ammonium Salts and Asparagin upon the Oxidation of Nitrites to Nitrates by Nitrobacter.

I. *Influence of Ammonium Salts.*

Winogradsky[1] was the first to observe that ammonium salts in very low concentration exerted a marked inhibiting action on the oxidation of nitrites to nitrates by Nitrobacter in mineral solutions. He found that when a little of a pure culture of the organism was seeded into a nitrite solution containing only ·0008 per cent. nitrogen as ammonium sulphate, the oxidation required twice as long to complete as in the nitrite solution alone. In the presence of ·015 per cent. ammoniacal nitrogen no oxidation of nitrite occurred even after months. He concluded therefore that ammonium salts behave as poisons towards the organism. Boulanger and Massol[2] were able to confirm these observations, but also found that when an ammonium salt was added to a solution in which the organism had already oxidised nitrite to nitrate, much higher concentrations were required to inhibit the action; ·009 per cent. ammoniacal nitrogen had no effect on oxidation, and even ·075 per cent. only postponed the completion of oxidation a few days. They therefore concluded that ammonium salts prevent the multiplication of the organism, but exercise very little influence on the actual oxidation when Nitrobacter has already abundantly multiplied.

The writer had already started some experiments along similar lines before the results of the two latter observers came to his notice. His object was to study the influence of ammonium salts upon the oxidation of nitrites in the presence of large inoculations of a fresh and active organism, and to observe whether an organism which had been already

[1] Article on Nitrification in Lafar's *Handbuch der technischen Mykologie,* 2nd edition.
[2] *Ann. Inst. Pasteur*, 17 July 1903, 18 March 1904.

accustomed to an ammonium salt could set up oxidation in a fresh nitrite solution containing ammonia.

Experiment I. This was arranged to show what effect the addition of an ammonium salt at the outset together with large inoculations of an organism, which had been previously grown in the entire absence of ammonia, would have on the oxidation of nitrite to nitrate. The culture solution used had the following composition, after Omeliansky:

Sodium nitrite	1·50 gram.
Ignited sodium carbonate	1·00 „
Monopotassium phosphate	0·50 „
Sodium chloride	0·50 „
Cryst. ferrous sulphate	0·40 „
Cryst. magnesium sulphate	0·30 „
Distilled water	1 litre.

30 c.c. portions of the above were sterilised in Erlenmeyer flasks of 125 c.c. capacity and divided into two sets, one receiving in addition 1 c.c. doses of a 3 per cent. ammonium sulphate solution. At this point there was present in each flask 9 mgs. of nitrite nitrogen, and in the ammonia series 6 mgs. of ammoniacal nitrogen also. The organism used for inoculation was taken from a culture in a nitrite solution of similar composition to the above, in which it had oxidised 120 mgs. of nitrite nitrogen to nitrate per 100 c.c. solution, the nitrite having been added in successive doses of 20 mgs. as the reaction for it disappeared. This organism had been at no period of its culture from the original arable soil in contact with an ammonium salt. The mixed liquid and sediment of this culture were well shaken and varying quantities inoculated into the above-mentioned solutions; three flasks containing nitrite and ammonium sulphate received 2, 2, and 5 c.c. inoculations, and four others containing only nitrite 1, 2, 4, and 5 c.c. inoculations. The cultures were incubated at a temperature of 30°. As the nitrite disappeared by oxidation in each series a fresh dose was added in the form of 1 c.c. of a 5 per cent. sodium nitrite solution; fresh additions of ammonia were also made to the ammonia series, in the form of 1 c.c. of a 3 per cent. ammonium sulphate solution. As the nitrite was oxidised in the latter set the ammoniacal nitrogen was determined by withdrawing 2 c.c. of liquid and Nesslerising, calculating the quantity of nitrogen as ammonia for the whole volume of liquid, which was measured on each occasion. The results of this experiment are given in Table III.

TABLE III.

Period of incubation	Controls Nitrite no Ammonia	Ammonium Sulphate series		
		2 c.c. inoculation *a.*	2 c.c. *b.*	5 c.c.
6 days	no NO_2 reaction	wk. NO_2 reaction	wk. NO_2 reaction	wkr. NO_2 reaction
6 days later	,,	no NO_2 reaction	no NO_2 reaction (3·9 mgs. N as NH_3 present)	no NO_2 reaction
7 days later	,,	no NO_2 reaction (7·60 mgs. N as NH_3 present)	—	wk. NO_2 reaction
6 days later	,,	no NO_2 reaction	—	no NO_2 reaction
7 days later	,,	no NO_2 reaction (11·0 mgs. N as NH_3 present)	—	wk. NO_2 reaction
9 days later	—	—	—	no NO_2 reaction (10·5 mgs. N as NH_3 present)
6 days later	no NO_2 reaction	no NO_2 reaction (26·2 mgs. N as NH_3)[1]	—	—
12 days later	,,	—	–	no NO_2 reaction (16·5 mgs. N as NH_3)[2]
59 days in all				

[1] =0·075 per cent. N as NH_3 in the solution. [2] =0·050 per cent. etc.

It is evident that at first there has been an inhibition of oxidation in the presence of ammonium sulphate, twelve days being required to complete it as against only six in the controls. When inoculation had been made with 5 c.c. of the culture oxidation was more rapid, as shown by the weaker nitrite reaction after six days. A determination of the residual ammonia in one flask at the end of the twelve days showed that there had been considerable volatilisation from the alkaline liquid, but there still remained ammoniacal nitrogen equal to ·013 per cent., but little short of the quantity stated by Winogradsky (·015 per cent.) to completely stop oxidation. In the third period after the addition of fresh nitrite and ammonium sulphate, oxidation was complete in all the series, though the ammoniacal solutions now contained ·025 per cent.

ammoniacal nitrogen. By successive additions of nitrite and ammonium sulphate, oxidation could be made to proceed as rapidly in the presence of ·075 per cent. ammoniacal nitrogen as in the controls containing only nitrite. One must conclude therefore that with a sufficiently heavy inoculation at the outset much of the inhibiting action of an ammonium salt can be avoided, although the organism had had no previous opportunity of habituating itself to ammonia; the inhibition is however still perceptible in the presence of ·013 per cent. ammoniacal nitrogen. The subsequent rapid oxidation of nitrite, in spite of increasing quantities of ammonia, confirms the results of Boulanger and Massol.

Experiment II. The object here was to observe the effect of adding ammonium sulphate to solutions in which oxidation of nitrite had occurred already several times. For this purpose two of the control solutions from the previous experiment, which had converted three successive doses of nitrite to nitrate, received an addition of fresh nitrite, together with 8 mgs. of ammoniacal nitrogen as ammonium sulphate. These two solutions had originally received 4 c.c. and 1 c.c. inoculations of the organism. A control solution without ammonia was also incubated. The result was as follows:

Date	4 c.c. culture and Ammonia	1 c.c. culture and Ammonia	Control Nitrite only
6 days	no NO_2 reaction (6 mgs. N as NH_3 present = ·02 p.c.)[1]	wk. NO_2 reaction	no NO_2 reaction
2 days later	—	no NO_2 reaction	—
4 days later	no NO_2 reaction (·038 p.c. N as NH_3 present)[2]	—	no NO_2 reaction
4 days later 16 days in all	—	no NO_2 reaction (·047 N as NH_3 present)[3]	—

[1] = 0·02 per cent. N as NH_3 in solution.
[2] = 0·038 etc. [3] = 0·047 etc.

In the first period, with 4 c.c. of the inoculating medium, nitrification was as rapid in the presence of ammonium sulphate as in the control, although there was half as much again ammoniacal nitrogen present as at the end of the first period in Experiment I. It is here evident

that, where the organism has already strongly multiplied, an addition of ammoniacal nitrogen in excess of that stated by Winogradsky to prevent all oxidation has had no inhibiting effect.

Experiment III. The object here was to observe whether ammonia salts inhibit oxidation by an organism which had previously been habituated to high concentrations of ammonium salts. The organism employed was the one cultivated in the ammonia series of Experiments I. and II. The cultures were all united, well shaken, and in every case only 1 c.c. used for inoculation. Six sterile solutions of nitrite were prepared as previously described and treated as follows:

1, 2, and 3 received 1 c.c. inoculation and 10 mgs. ammonium sulphate.
4 „ only inoculation.
5 „ only ammonium sulphate.
6 „ no addition.

For the actual experiment only 1, 2, 3 and 4 are of interest. The flasks were incubated as before at 30°, and the ammonium sulphate and nitrite were renewed as in Experiment I., the ammonia present from time to time being determined in the manner there described. The results are shown in Table IV.

In order to bring out the significance of the results it is necessary to compare them with those obtained in Experiment I. There the presence of ·013 per cent. ammoniacal nitrogen doubled the time required for controls, here there has been no inhibition even in the presence of double the quantity of ammoniacal nitrogen, namely ·026 per cent., and with a weaker inoculation of the organism.

It is then evident that the nitrobacter, by previous cultivation in solutions with gradually increasing ammonia content, has acquired the property of withstanding the deleterious effect of the latter upon its growth.

In this series oxidation in the presence of as much as ·119 per cent. ammoniacal nitrogen has kept pace with the controls. Oxidation was finally stopped in both series alike, not by the ammonium sulphate concentration, but by the accumulation of the nitrate produced. A determination of the nitric nitrogen in the latter showed an amount equal to ·95 per cent. sodium nitrate.

TABLE IV.

Period of incubation	Culture 1. with Ammonia	Culture 2. with Ammonia	Culture 3. with Ammonia	Culture 4. (Control) Nitrite only
Dec. 24–Jan. 2 9 days	no NO_2 reaction (7·8 mgs. N as NH_3)[1]	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction
Jan. 2–8 6 days later	—	no NO_2 reaction (15·8 mgs. N as NH_3)	wk. NO_2 reaction	,,
Jan. 2–11 3 days later	—	—	no NO_2 reaction (15·3 mgs. N as NH_3)	—
Jan. 8–13 2 days later	—	no NO_2 reaction (23·1 mgs. N as NH_3)	—	no NO_2 reaction
Jan. 11–16 3 days later	—	—	no NO_2 reaction (28·5 mgs. N as NH_3)	—
Jan. 13–19 3 days later	—	no NO_2 reaction (33·3 mgs. N as NH_3)[2]	—	no NO_2 reaction
Jan. 19–31 12 days later 38 days in all	—	str. NO_2 reaction (40·5 mgs. N as NH_3)	str. NO_2 reaction (35·0 mgs. N as NH_3)	str. NO_2 reaction

[1] = 0·026 p.c. N as NH_3 in the solution. [2] = 0·119 etc.

Loss of Nitrogen from the Nitrite Culture Solution containing Ammonium Sulphate.

It was thought that there might be a reaction between the nitrite and the ammonium salt leading to a loss from the solution in the form of nitrogen gas. To determine if this were so the solutions 5 and 6 were incubated with the cultures of Experiment III., and the ammonia and nitrite determined in 5 and nitrite in 6 after nine days. The ammonia was also determined in culture 1. The results were as follows:

Number	NH_3N	NO_2N
1.	7·80 mgs.	—
5.	8·0 ,,	8·80 mgs.
6.	—	8·90 ,,

There was evidently no loss of nitrogen by reaction between the nitrite and ammonia in flask 5. There was originally 10 mgs. ammoniacal nitrogen present, so that the only loss was due to volatilisation of ammonium carbonate from the alkaline liquid, amounting to 20–22 per cent. of the quantity added. That the loss was of this nature was shown by a determination of the alkalinity of 1, 5, and 6, cochineal being used as indicator.

To neutralise	1	required	3·3	c.c. N/10 acid.
"	5	"	3·4	"
"	6	"	5·8	"

The reduction of alkalinity in 1 and 5 indicates loss of ammonium carbonate from the solution. The maximum amount of ammonium sulphate nitrogen converted to carbonate by the sodium carbonate present would be 8·30 mgs., or 83 per cent. of the quantity at first added. It may be assumed therefore that in the solution used for the three experiments a part of the ammonia, up to ·027 per cent., must have been in the form of carbonate, but no experiments have been made to decide the point as to whether the carbonate and sulphate of ammonium affect the growth and oxidising power of nitrobacter in different degree.

II. *Influence of Asparagin.*

Winogradsky has stated that asparagin in a concentration of ·70 per cent. prevents the oxidation of nitrite to nitrate.

Some experiments have therefore been made in the same manner as with ammonia, to determine whether by strong inoculation of the organism the inhibiting action of asparagin can be lessened or removed.

Experiment I. The object here was to determine whether a strong inoculation of an organism which had never been in contact with asparagin would bring about oxidation of nitrite in the presence of the above-mentioned asparagin concentration, namely ·70 per cent. of the solution.

The procedure was similar to that adopted for Experiment I. of the ammonia series, the organism used being the same also. The results are shown in Table V. A marked slowing of oxidation is evident at the outset, the controls having oxidised three successive nitrite doses before oxidation was completed in the asparagin series. The first oxidation required 19 days, but after a fresh addition of nitrite, oxidation was as rapid as in the controls. A determination of the asparagin in the culture inoculated with 5 c.c. of organism showed that at the end of

TABLE V.

Period of incubation	Controls Nitrite only	Asparagin ·72 p.c. of the solution		
		1 c.c. inoc. *a.*	1 c.c. inoc *b.*	5 c.c. inoc.
6 days	no NO_2 reaction	str. NO_2 reaction	str. NO_2 reaction	mod. NO_2 react.
6 days later	,,	mod. NO_2 react.	mod. NO_2 react.	wk. NO_2 reaction
7 days later	,,	no NO_2 reaction (added NO_2 sn.)	no NO_2 reaction (added NO_2 sn.)	no NO_2 reaction (added NO_2 sn.)
7 days later (26 days in all)	,,	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction (asparagin present = ·71 p.c.)

the experiment the original quantity was still present. The same conclusion must be drawn as for the first experiment with ammonia, namely that asparagin inhibits growth but that with a large inoculation of the organism the effect can be largely obviated.

Experiment II. The object was to observe the effect of adding asparagin to solutions in which the oxidation of nitrite had occurred several times. For this purpose two control solutions from Experiment I. in which three successive doses of nitrite had been oxidised, received 2 c.c. doses of a 10 per cent. asparagin solution [giving a concentration of

TABLE VI.

Period of incubation	Control	Asparagin series	
		5 c.c. inoc.	2 c.c. inoc.
6 days	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction (asparagin determined = ·705 p.c.)
2 days later	—	further asparagin added	—
7 days later	no NO_2 reaction	wk. NO_2 reaction	—
3 days later	—	no NO_2 reaction (asparagin determined 1·115 p.c. ·005 p.c. ammonia)	—

0·71 per cent.], together with a fresh dose of nitrite, and were incubated with a control which received no asparagin but fresh nitrite at the same time. The results of this experiment are shown in Table VI. Here there was no slowing of oxidation in the presence of asparagin at the outset, the two cultures containing that substance having oxidised as rapidly as the control, namely in six days. The asparagin was then determined in one of them and found to be identical with the amount originally added. To the other culture a fresh dose of asparagin was added and more nitrite, with the result that oxidation was complete in 10 days as against 7 days in the control. A determination of the asparagin showed 1·115 per cent. and a little ammonia amounting to ·005 per cent. to be present, the latter being due to a slight attack on the asparagin by foreign organisms conveyed in the not perfectly pure inoculation of nitrobacter. One must conclude from this experiment that, when the organism has previously multiplied vigorously in a pure nitrite solution, the subsequent addition of asparagin in amount equal to what had markedly inhibited oxidation in Experiment I., has no effect in checking oxidation.

The same result has therefore been obtained as in Experiment II. of the ammonia series, and the same conclusions may be drawn from the two asparagin experiments as from the Experiments I. and II. of the ammonia series, namely that both substances have an inhibiting influence upon the growth of nitrobacter, but this effect disappears when the growth of nitrobacter is specially vigorous or has become habituated to the inhibiting substance.

The conclusions derived from these various observations on nitrification may be summarised as follows:

1. That carbonates are not the only substances in the soil which serve as bases for nitrification, since a marked nitrification of an ammonium salt can be brought about in the presence of ferric hydrate, either in the freshly precipitated state or as "iron rust." In solutions nitrification is not completed with this substance, probably because the ferric nitrite or nitrate formed dissociates and the solution becomes acid.

2. That neither kaolin nor modelling clay serves as a base for nitrification.

3. That the double ammonium combination formed by the absorption of ammonium salts by modelling clay can most probably be nitrified in the absence of any base, but that the corresponding combination with peat undergoes no nitrification in the absence of a base.

4. That the function of the base in nitrification is to form ammonium carbonate which is alone directly nitrifiable, and that the facility with which nitrification is set up by different carbonates depends upon the rapidity with which they can react with a neutral ammonium salt to produce ammonium carbonate. This reaction is greater with magnesium carbonate than with calcium carbonate, but is almost absent with copper carbonate, a result which is not due to a poisonous action on the organism.

5. That ammonium salts and asparagin inhibit the oxidation by nitrobacter of nitrites to nitrates, but this action can be largely obviated by

(*a*) abundant inoculation of the organism;

(*b*) allowing the organism to multiply before addition of the ammonium salt or asparagin;

(*c*) inoculating with an organism which has become habituated in previous culture to ammonium salts or asparagin by gradually increasing the concentration of the latter substances.

THE HYBRIDISATION OF CEREALS.

By JOHN H. WILSON, D.Sc., F.R.S.E.,

Lecturer in Agriculture and Rural Economy in the University of St Andrews.

With Plate I.

THE purpose of the present paper is to give some account of experiments with oats, wheats, and barleys, carried out personally at St Andrews during the past five years.

The Hybridisation of Oats.

The production of new varieties of Oats by crossing is not a very difficult matter. A fair knowledge of floral structure suffices to lead to successful methods of manipulation.

Some experimenters insist that they have succeeded in raising new varieties by simply sowing two distinct varieties together, leaving them to nature, and gathering the grain. If success is ever achieved by this method it can only be as the result of a series of fortunate accidents of very unusual occurrence. The writer's experience leads him to believe that before natural crossing could take place a number of obstacles would have to be overcome. Flowers of the different varieties would have to open simultaneously. The anthers of certain flowers would have to prove abortive, or their pollen infertile. Pollen would require to be carried from the anther of one kind to the stigma of the other. In spite of many obstacles, however, instances of genuine natural crossing of cereals are recorded. Shirreff[1] affirms that a natural cross-bred wheat appeared in his plots. Dr Charles E. Saunders[2], of the Central Experimental Farm, Ottawa, records the finding of a natural cross between Polish wheat and some other variety in a plot of Polish

[1] Shirreff, *Improvement of the Cereals*, p. 46.

[2] C. E. Saunders, *American Breeders' Association*, Vol. I., 1905, p. 137.

wheat. Rimpau[1] in the course of his observations found a number of naturally crossed wheats, barleys, and oats, the wheats being most numerous.

In experimental crossing it is obviously quite essential to remove the anthers at an early stage from the florets to be pollinated, and it is also a wise precaution to protect the prepared florets both before and after the application of the pollen. Small bags are commonly used for the purpose of protection. The writer has found specially constructed glazed boxes convenient. The outer floret of the spikelet was invariably taken for pollination in the present experiments.

The following crosses were successfully carried out in 1901:

Goldfinder with Potato	4 grains secured.
Goldfinder with Waverley	3 " "
Goldfinder with Black Tartarian ...	2 " "
Waverley with Black Tartarian ...	1 grain "
Black Tartarian with White Canadian	1 " "
Black Tartarian with Abundance ...	1 " "

It would serve no good purpose to dwell on details of the failures met with, for it is probable that they were very largely due to misfortune or inexperience. So far as the writer is aware it should be possible to cross any variety of oat with any other if due skill be employed. Nevertheless there are circumstances which tell against success. In 1903 an effort made to cross a considerable number of varieties resulted in complete failure. The non-success was attributed to the effect of over-stimulation by a nitrogenous fertiliser. The season, however, was very unpropitious. It is a well-known circumstance that when plants are in too active a condition of vegetative growth they are unlikely to set fruit well.

The crossed grains, twelve in number, were sown singly in small flower-pots, and when the first green leaf was about an inch above the ground they were planted out. One failed to germinate. The remaining eleven plants grew to unusual size, outstripping the examples of the parent varieties alongside. The latter were grown in a manner quite similar to the hybrids, except that they were sown in the ground direct. The excessive vigour of the hybrids could not be accounted for by any help that transplanting might give; it seemed clearly to be due to inherent qualities, and to afford another instance of the well-known phenomenon of enhancement of vitality consequent on crossing.

[1] Rimpau, *Landw. Jahrb.*, 1891.

Descriptions of Parent Plants and Hybrids.

The hybrids between black and white oats have been found to offer most features of interest. Attention is directed to three of them now, viz. Goldfinder × Black Tartarian, Black Tartarian × White Canadian, and Black Tartarian × Abundance, the remaining one, Waverley × Black Tartarian, being left for discussion later (p. 79).

It will be seen that in the first-named cross the Tartarian is the pollen parent, whereas in the next two the Tartarian is the seed parent. It will be shown in the sequel that so far as the transmission of the colour of the grain goes it is immaterial whether Tartarian occupies the one place or the other. Opportunity was not given of showing whether in the transmission of the form of the ear, etc., a similar state of affairs existed. In the meantime we may pretty safely assume that reciprocal crossing would give identical results. Such has been found to be the case in wheats by other observers. It is by no means the case, however, that all reciprocal crosses are identical, in groups outside of the cereals, at any rate.

Goldfinder.

The parentage of this oat is given by the raisers, the Messrs Garton, as follows: (White Canadian × Yellow Poland) × Winter.

However carefully the new breeds are selected and fixed there must naturally be far greater likelihood of variation in recruits, so to speak, than in veterans which have stood the test of time. The stock of Goldfinder was, it is believed, quite above suspicion of admixture, but the records of the present experiments point to a tendency to variation in the form of the ear and some instability in the varying number of the grains in the spikelet, four being occasionally found. The ears of the plants used as parents were strongly inclined to hang to one side, but could not be classed as other than pyramidal. The grains were golden.

Black Tartarian.

This old standard oat is characterised by two marked features, the one being the unilateral form of the ear, and the other the "black" colour of the "grain," that is, the colour of the pales which enclose the grain proper. The spikelets consist of two florets, a third being occasionally seen. Awns may be numerous, few, or none. The ear is dense, this being due to the upright growth of the branches. When the spikelets interlock, as they usually do, more or less, the ear looks very narrow.

Goldfinder × Black Tartarian.

Of this cross one grain was good, the other very poor. The latter was solid enough at its basal (germinal) end, but shrivelled and empty-looking above. Notwithstanding the deficiency in store of starch, the seedling from this poor grain grew fairly well. It bore the appearance of having been checked at the commencement of growth, and it lacked the vigour of its neighbour. Its strongest stem rose to 6 ft. 6 in. There were four other strong stems and 17 weaker ones, besides a crowd of weakling late shoots. The longest ear was 18 inches.

The plant from the good grain bore eight strong stems and nine weaker ones, but no late ones. The tallest attained the height of 6 ft. 6 in., and the longest ear was 18 in. This ear (Fig. 1) was composed of 296 spikelets, and, allowing for grain lost, it was computed to have borne 657 grains. Neglecting the difference in vigour, the two plants were alike in all respects.

The ears showed a blending in form between the two parental types, being somewhat elliptical in outline and considerably flattened one way. The grains also were intermediate, being rich brown in colour. A third grain in the spikelet appeared frequently. Awns were hardly ever developed.

White Canadian.

The grain used under the name of White Canadian was taken from a commercial sample, and its history cannot be traced. There is much reason to believe that this sample contained more than one variety, and therefore considerable uncertainty exists regarding the authenticity of the seed parent. So far as the present enquiry is concerned, the dubiety is not of much consequence. The tallest plant was 5 ft., the ears pyramidal, spreading, the longest one 11 in. Three-grained spikelets occurred, but they were not numerous. Awns were absent.

Black Tartarian × White Canadian.

The hybrid grain was small. In spite of this the plant grew well, the tallest stem reaching over 6 ft. Ten strong stems and 12 weaker ones were produced. The longest ear was 18 in. The form of the ear was fairly intermediate, spreading considerably, but decidedly one-sided. A third grain was frequently present in the spikelet. The grain ripened to a rich brown colour. Awns were absent.

Abundance.

The pedigree given by the raisers, the Messrs Garton, is as follows: White August × White Swedish.

The ear of this variety is open pyramidal, and the grain is white. Three-grained spikelets are of common occurrence, and awns are very exceptional. The tallest plant in the plots was 5 ft. 7 in., and the longest ear 11¼ in.

Tartarian × Abundance.

The single grain secured of this cross was a good one, and the plant grew well, producing 11 strong stems and 11 weak ones. The tallest reached the height of 6 ft. 4½ in. The longest ear was 15½ in. The ears were somewhat open, but with a marked tendency to the one-sided type. Three-grained spikelets were of fairly common occurrence. The ripe grain was deep brown. No awns were present.

The hybrids were very late in ripening, and when still unripe a gale beat against the frame of the net under which they were placed, bending the majority of the stems and breaking a good many. Immediate steps were taken to straighten and support the bent and half-broken ones, but it was manifest that the crop was lessened very considerably by the accident.

Selection of the Hybrid Seed.

In spite of the damage done by the gale it was found possible to select much fine seed for further cultivation. The system of selection adopted was as follows: The grains were selected in the first place according to their position in the spikelet, whether outer, mid or inner. The grains were taken almost without exception from spikelets composed of three grains. The grains so selected were mixed in their several classes, and sets of hundreds, sometimes fifties, picked out, commencing with the finest samples. Twelve thousand of these grains were sown in dibble holes made six inches apart each way.

The vigour of the hybrids was again in evidence in the fine growth made by the entire crop. Everything went well for a time, but the extremely bad weather of the autumn of 1903 wrought great destruction over the whole plot. The plants were usually over 6 feet high, but they lodged and much grain was thus lost. Harvested in a sodden state, the heads of each plant were tied together, and the product of the rows made into separate bundles and hung up to dry. The ears were

so seriously damaged as to preclude the possibility of making comparative observations on their form. This was a very regrettable loss, because it was obvious that very interesting variation had taken place in respect of this character, by the occurrence in many of the Black Tartarian crosses of examples with black grains and spreading, pyramidal, not one-sided, ears. It was impossible, however, to discriminate between different shades of brown, which in perfect grain might have enabled one to separate such plants as might have taken after the hybrid parent from those which might have resembled the Tartarian grandparent. The so-called "black" of the latter is merely a very deep brown, and well-matured hybrid grain and badly-matured Tartarian grain are of the same tint. It is convenient to designate all that take after the Tartarian grandparent in colour characters as "black," and all that take after the other grandparents—whatever be the shade of gold, straw-colour or whitish—as "white."

Account was kept of the numbers of blacks and whites in the three sets, and the subjoined table drawn up:

Goldfinder × *Black Tartarian.*

(From the two plants of the first generation.)

Grains sown.	Plants harvested.	Black-grained plants.	White-grained plants.	Ratio of black and white.
(1) 1000	567	433	134	3·23 : 1
(2) 900	566	415	151	2·75 : 1
Black Tartarian × *White Canadian.*				
890	532	379	153	2·48 : 1
Black Tartarian × *Abundance.*				
600	274	209	65	3·21 : 1.

It is perfectly obvious from the above figures that a fixed numerical relationship exists between the black and white derivatives of hybrid oats in the second generation, in respect of the colour of the grain, the proportion of the former to the latter being very nearly 3 : 1. The totals are sometimes over, sometimes under, the exact terms of this ratio. In all cases the plants saved from the century sets were both black and white. In some cases the black plants from the set were exactly three times as many as the white. In other cases there was a wide departure from this proportion, but when a sufficiently large number of sets were added together the general average approached the simple ratio closely.

In other cereals the characters which have been found by Spillman, Biffen and others to be subject to Mendelian segregation are such as the form of the ear, the colour of the grain, the colour of the chaff, the presence or absence of awns, etc.

If the hybrid oats conformed to Mendel's law it was obvious from the tables given that the blacks should be dominant and the whites recessive. Experiments were proceeded with to prove this.

As already stated, it was impossible to separate the blacks from the browns in the specimens dealt with above. Samples were taken from individual plants (often from single heads). The grains from the blacks were sown in rows of 100 or 50 side by side, and the white derivatives of the same origin were sown in a similar way in the near neighbourhood.

No black oats were to be found among the white ones at harvest, that is to say, the expectation based on Mendel's law was fully realised, and the recessive types demonstrated to be fixed as regards this character.

It was otherwise with the rows of blacks. Here a large number of white-grained plants were found dotted promiscuously in certain of the rows, while other rows were entirely composed of black-grained plants. It was clear that grain from plants repeating the hybrid character had been sown in the former, while in the latter the grain had been from plants which were pure extracted dominants.

Experiments with the Grains of Individual Spikelets.

An effort to test whether the grains of individual spikelets varied among themselves as bearers of hereditary qualities; in other words, whether they would produce either dominant or recessive characters only, or both. This experiment was not tried in 1903 when the bulk of the hybrid grain was sown, but fortunately a few ears—very poor ones—of Goldfinder × Black Tartarian and Black Tartarian × Abundance had been kept. Twelve spikelets of each kind were secured, and these yielded sufficient seed to show at least general results. The spikelets all bore three grains, the innermost (uppermost) one being in most cases very small. The grains were all rich brown.

The following method of sowing was followed:—The grains of each spikelet were sown in series, the largest marked I, the mid one II, and the smallest III. The seed was not only a year old, it was also very late in being sown. The plants made very indifferent growth, and were harvested late in poor condition. It was impossible to say in several

cases whether the grain gathered was black or white, and this doubtful grain could not be counted.

The subjoined table, compiled from the available grain, shows the distribution of colour over the several sets, each plant of course being found to bear either black or white grain:

Goldfinder × *Black Tartarian.*

Position in spikelet...	I.		II.		III.	
Colour...	Black	White	Black	White	Black	White
Spikelets						
1	×	—	×	—	—	×
2	×	—	×	—	—	×
3	—	×	×	—	—	—
4	×	—	—	×	—	—
5	×	—	—	×	—	×
6	×	—	×	—	×	—
7	×	—	×	—	—	×
8	—	—	×	—	—	×
9	×	—	—	×	×	—
10	×	—	—	×	×	—
11	—	×	—	×	×	—
12	×	—	—	×	—	×

Black Tartarian × *Abundance.*

Spikelets	I. Black	I. White	II. Black	II. White	III. Black	III. White
1	×	—	×	—	×	—
2	×	—	—	—	—	—
3	×	—	×	—	—	×
4	—	—	×	—	—	—
5	×	—	—	—	×	—
6	—	—	—	×	—	—
7	×	—	—	×	×	—
8	×	—	—	×	—	×
9	—	—	×	—	×	—
10	×	—	—	—	×	—
11	×	—	×	—	×	—
12	×	—	×	—	×	—

The total number of plants bearing either black or white grains can be tabulated as follows:

Goldfinder × *Black Tartarian.*

	I.	II.	III.
Black............	9	6	4
White	2	6	6

Black Tartarian × *Abundance.*

	I.	II.	III.
Black............	9	6	7
White	0	3	2

Poor as the material was it proved to be sufficient to show that in the original hybrid the grains of individual spikelets varied among themselves in respect of the latent characters they were endowed with, and that no account need be taken of the position of the grains in the ear so far as the distribution of hereditary traits is concerned.

The time had now come when it was desirable to grow larger quantities of certain of the varieties. Accordingly selection was made of a varying number of plants that were regarded as alike in the several rows of the third generation, which had been sown from separate hundreds (or fifties) as described. The grain of the plants chosen from a row was mixed together, and a quantity of it sown by hand about 3 inches apart in drills of the usual kind in the field.

The history of a number of the plants of which continuous records had been kept to the fourth generation is summarised in the annexed table:—

	Position of grains in spikelet of 1st generation	No. of 1st generation sown	Segregation of plants of 2nd generation harvested		Single plant chosen for 3rd generation		No. of grains sown	No. of plants harvested	No. of similar plants chosen for 4th generation	No. of sheaves of 4th generation	Character of plants of 4th generation
			Blacks	*Whites*	*Dominant*	*Recessive*					
											Goldfinder × Black Tartarian.
A	First	100	54	14	—	×	100	78	Many	14	Heavy, drooping, one-sided ears, to be regarded as fixed. Much stronger than Potato, and about a week later.
B	First	100	38	13	—	×	50	43	33	12	One-sided ears; none pyramidal. Held to be fixed. Not early.
C	Second	100	42	17	—	×	50	34	9	5	Fine, tall, one-sided grain. A few pyramidal ears.
D	Third	50	20	5	—	×	50	35	34	25	One-sided, open and close, with a few distinctly pyramidal.
E	Second	100	37	16	×	—	50	24	17	7	Open one-sided, uniform, with an occasional pyramidal.
F	Second	100	42	14	×	—	50	30	25	17	Close or somewhat open one-sided. A few pyramidal. (One white-grained plant.)
G	Second	100	42	14	×	—	100	68	48	17	All pyramidal, spreading or slightly compressed. Held to be fixed.
H	Second	100	54	15	×	—	50	38	29	8	Mixture of one-sided, hybrid and pyramidal.
I	First	100	54	12	×	—	50	29	25	9	Mixture of one-sided, hybrid and pyramidal. Very tall, and extraordinarily late.
J	First	100	43	17	×	—	50	36	24	16	Open one-sided and pyramidal. Not so tall as usual.
K	First	100	53	17	×	—	100	78	60	18	Mixture of close one-sided, hybrid and pyramidal.
L	Second	100	49	19	×	—	100	78	56	8	Open and close one-sided. None quite pyramidal.
M	Third	100	54	12	×	—	100	59	31	12	Mixture of one-sided, hybrid and pyramidal.
											Black Tartarian × White Canadian.
N	First	100	54	18	×	—	100	75	67	7	The great majority pyramidal. A number of fairly close one-sided ears.
											Black Tartarian × Abundance.
O	First	100	47	12	—	×	100	51	42	6	Mixture of pyramidal and open and close one-sided. The pyramidal greatly in the majority.
P	Second	100	18	7	×	—	100	70	64	6	All pyramidal and quite uniform.

The above table is interesting in showing clearly the difficulty in selecting when one has not been fortunate enough to get a full knowledge of the Mendelian characters of the plants worked with. So far as colour of the grain is concerned, the only point to notice is that one white-grained plant appeared in F, the bulk of which amounted to seventeen sheaves. It is in the highest degree probable that a single white seed had been sown amongst the black by accident.

In A and B the results are white forms with a one-sided ear, now fixed. In C and D, also white forms, there seems to be still a tendency to vary, a few pyramidal ears appearing amongst the one-sided forms. G and P are examples of black varieties with pyramidal ears, now fixed. In the other varieties there is ample room for further selection.

In I a feature of unusual interest was noted. All the plants, regardless of the form of their ears, were absolutely uniform in one particular, viz. distinct lateness. The row had to be left to ripen when all the others had been harvested.

In one of the experiments with Tartarian × Abundance, the line of descent of the plants was different from that followed by those in the above table. To begin with, four rows of grain from the original hybrid yielded the following:—

	Blacks	Whites
A	18	7
B	49	9
C	33	17
D	47	12
	147	45

Grain from a single plant of each of the "blacks" of A, B, C, D was chosen and sown, and the following plants harvested:

	Blacks	Whites
A′	63	20
B′	30	11
C′	27	10
D′	22	8
	142	49

The totals in both tables show a close approximation to the Mendelian ratio. The second table points unmistakably to the fact established by Mendel that certain of the progeny of the third generation repeat the dual character of the second, and do not breed true. The grains of A′, B′, C′, D′ were rich brown, and that feature in itself betokened their hybrid character.

The whites from A′, B′, C′, D′ were mixed and sown in the field so as to yield eight sheaves. It was found that about a dozen plants with black grains and pyramidal ears appeared in the lines. Their presence could only be accounted for by accidental introduction of black seed. Although great care had been exercised to prevent mixture, it was not impossible that a bleached grain or two might have escaped notice in the course of selection. There is no reason at all to suppose that the route taken in descent had anything to do with the appearance of the blacks amongst the white recessives.

Waverley × Black Tartarian.

As already stated, this hybrid has not conformed to rule. The hybrid could not be distinguished from Waverley unless by its greater height and general vigour. The open pyramidal form of the ear was the same. The best ear was 13 in. long, and the height of the plant 6 ft. 5¼ in. The grain of this plant was sown along with that of the hybrids above described. The result was remarkable. The plants saved from the wreckage of 1903 showed that a trace of the blood of the Black Tartarian was in their constitution, for a distinct black form appeared amongst the normal Waverley-like plants. No other types appeared except these two. The black one had a spreading pyramidal ear. It was represented by remarkably few plants. Many examples of both types were noted to be over 6 ft., the tallest in the normal form being 6 ft. 3½ in., and in the black 6 ft. 5½ in.

The number of Waverley-like plants lifted was not recorded, but all the black ones were carefully selected out and counted. Judging from the average number of plants of the other crosses saved and counted, it may be safely conjectured that the number of both lifted would not be less than one-half of that sown. On this basis the subjoined table has been framed:—

Waverley × Black Tartarian.

Proportion of Black-grained to White-grained Plants.

Position of grains in spikelet	No. of grains sown	Minimum no. of plants saved (conjectural)	Actual no. of blacks present
Outer	700	350	10
Mid	300	150	5
Inner	200	100	3
Totals	1200	600	18

Keeping in mind that both whites and blacks suffered diminution in the same degree, it is obvious from the above table that the number of black derivatives does not approximate at all to the Mendelian ratio. It may be described as a case in which the pollen parent has been for some unexplainable reason almost but not quite impotent.

The production of black-grained plants from all three grains of the spikelet is a point of interest in connexion with what has already been noted regarding this matter.

Next year 350 white grains were sown in the plots. At harvesting there were probably about 250 lifted, and all were of the original Waverley-like type except *two*, which were of the pyramidal black type.

In the following year (1905) such a number of white grains was sown as to yield eight or nine sheaves at harvest. *No example* of the black form was found in the lot.

Grains of the black oats were sown in the plots. Of the 53 plants harvested 34 were found to be of the black type, and the remaining 19 the white type. The white plants were conspicuously the stronger. No one-sided examples of either type were found. The number grown was scarcely large enough to ensure statistical certainty, but at present one may be justified in assuming that the black forms derived from the white ones in very small numbers, themselves give off white forms in relatively large numbers. The suggestion of the possibility of the black-grained plants being in this case of the nature of recessives is thus done away with.

Referring in conversation to the peculiarities of the above hybrid, Mr John Garton informed me that he had met with anomalous cases in some measure corresponding to the above. All who have had long experience in crossing must have seen instances which are exceptions to the rule. It is well to remember that the plants we work with are not built up after all with mathematical regularity, but that they are the outcome of developmental processes acting in inconceivably diverse ways and through long ages of time, and that therefore there need be no surprise if they do not always fall in with the rigid and narrow formulae of the statistician. The practical lesson to be learned from this case is that it is possible that good things may be lost by too close adherence to arbitrary rules of selection.

Experiments in Crossing Wheats.

Several crosses were carried out with wheats, viz., Red King × Red American, Red King × Scotch Bethlehem, and Red King × Rood

Koren. The most interesting of these is the last mentioned and it alone will be described.

Red King.

The raisers of this wheat, the Messrs Garton, give the following as its pedigree:—(Lincoln Red × Michigan Bronze) × Waterloo. The straw of this variety is of medium length and particularly strong. The ears are long and compact (Fig. 2, *a*).

Rood Koren.

This bearded wheat (Fig. 2, *c*) was received from a friend who stated that it had come from the Orange Free State. Request for information being made regarding it to Mr William Macdonald, M.S., Agr., Editor of the *Transvaal Agricultural Journal*, the following particulars have been kindly given by him:—

"The Rood Koren was imported eight or ten years ago from Canada by Mr A. C. Macdonald, Assistant Director of Agriculture, and is probably a strain of Red Egyptian. It is a very hardy variety, and is widely used for milling purposes. Besides being considered one of our very best flour wheats, it is more or less rust-resistant."

Mr J. Burtt-Davy, Agrostologist and Botanist to the Transvaal Department of Agriculture, obligingly adds the following note:—"I may point out that of some 200 varieties of wheat tested by us last season, only one proved rust-resistant. This was the 'Red Egyptian,' obtained from Vilmorin-Andrieux et Cie, Paris."

The Rood Koren grew from 2 to 3 feet high in the plots. Some examples in special ground were nearly 4 feet. The length of the ears is $2\frac{1}{4}$ to $2\frac{3}{4}$ inches, or including awns about 5 inches. The awns are from $2\frac{1}{2}$ to $3\frac{1}{2}$ inches. The average number of grains in the ear is 40. The greatest number of ears produced by one plant was 28.

Rood Koren has been singularly free from rust in the plots, and its progeny have been far less subject to the disease than those of the other crosses named above.

Red King × Rood Koren.

Four grains were secured of this cross in 1902. All four grains were from one ear of Red King. They were sown in the ground in November of the same year. One, a poor grain, failed to germinate, the other three grew apace. On July 2nd, 1903, it was noticed that

while two plants of the pollen parent (Rood Koren) were in flower, and all the plants of the seed parent had their ears still hidden within the sheaths, all the ears of the three hybrid plants were clear of the sheaths. The hybrids thus showed an intermediate condition in respect of the period of shooting into ear.

The hybrids were harvested about Oct. 6th. All three were identical in general characters. The tallest of each were as follows: A, 4 ft. 4 in.; B, 4 ft.; and C, 4 ft. 4½ in. The number of ears respectively were 19, 21, and 30. The longest ears in all were 3⅝ in., a common length being 3 in., and the longest awns (projecting from the apex of the ear) 1 in. The ears were square and compact (Fig. 2, *b*), and the straw much more slender than in Red King.

The average numbers of grains for the ten best ears of each were as follows: 54·9, 58·6, 62·3, giving a common average of 52·6.

Many grains of each were sown on 11th Dec., 1903. During the following summer it was again noticed that the hybrids showed an intermediate condition in the time of flowering, in many cases at all events.

The plants of the second generation showed the usual breaking up into many forms. Four distinct types could be easily recognised, viz. short-eared, long-eared, awnless, and awned (Fig. 3). When harvested the progeny of each original plant, A, B, C, was looked over and the awnless separated from the awned; then the same plants were looked over again, and, regardless of the awned or awnless condition, the short-eared separated from the long-eared. The subjoined table shows the proportions of the respective stocks, and the totals for all three:—

Red King × *Rood Koren.*

	Awnless	Awned	Short-eared	Long-eared
A.	164	58	165	57
B.	255	86	240	101
C.	245	63	238	70
Totals	664	207	643	228

The totals point unmistakably to Mendel's ratio of 3 : 1, the awnless being almost three times as many as the awned, and the short-eared occupying a similar relationship with the long-eared. These results are corroborative of previous ones got by other observers. The awned and the long-eared being recessive characters, one expects varieties possessing them to breed true to these characters.

It was noticed that there was very little variation indeed in the form of the ear of the long-eared types, both awned and awnless falling into one class. It was otherwise with the short-eared types. These could with considerable accuracy be classed into two sub-groups—longer and shorter. The following table, framed from the material available, shows the proportion of these types, their awned or awnless condition being taken into account:

Short-eared Types.

	With awns		Without awns	
	Longer	Shorter	Longer	Shorter
A.	30	13	78	44
B.	40	10	62	20
C.	34	19	188	67
	104	42	328	131

The totals here seem to point to the existence of Mendelian proportions in an inner circle. It would be unsafe, however, to press the matter further, the distinction to be drawn between the two sections—longer and shorter—not being very clearly defined.

A few plants of the short-eared type bore remarkably dense ears.

The grain from the three sets, A, B, C, was separated with reference to the length of ear and the presence or absence of awns, and the parcels under the respective heads mixed together for sowing. The examination of the crop showed that the Mendelian anticipations were fully realised. The plot containing the lot which embodied the two recessive characters, long ears with awns, presented the most uniform appearance, the ears being almost entirely long and awned. In the plot sown with grain from long but awnless ears, the ears were almost all long, and the majority were awnless. The plot sown from short-eared awned specimens was composed virtually of entirely awned plants, but the form of the ears was exceedingly variable, few, however, being long. The plants derived from the short awnless ears were of all types, awned and awnless, long and short, the long-eared and the awned being greatly in the minority.

Grain from a single short awnless ear was sown in a row by itself and the crop was found to be composed of every type, thus giving a complete repetition of the forms which occurred in the second generation. This result clearly emphasised the fact that the short awnless plants retain the hybrid characters and do not breed true.

The Hybridisation of Barleys.

The experiments on the crossing of barleys were commenced in 1903. Standwell was chosen as seed parent and crossed with other varieties of barley, viz. (*a*) a six-rowed variety which appeared amongst the Rood Koren wheat in the plots, and for convenience now designated Stranger, (*b*) Zero, (*c*) Egyptian, and (*d*) Bere.

Standwell Barley.

This variety (Fig. 4 *et seq.*, *a*), as stated by the raisers, the Messrs Garton, is a cross between Fan and Golden Melon. It is two-rowed, strong-growing, and early.

Zero Barley.

This interesting hybrid, also sent out by the Messrs Garton, has the following pedigree: (Winter × Fan) × Swedish. It is six-rowed and a distinct variety in every way. At an early stage of growth the foliage is distinctly dwarf, compared with either Standwell or Stranger, and it is the latest of all the varieties at present in question in showing ear.

Six-rowed Barley (Stranger).

This variety (Fig. 4, *c*) came up in the experimental plots, and was possibly brought from the Transvaal with the Rood Koren wheat. It bears much resemblance to Zero, but its fresh foliage, while of the same shade of light green, is much stronger and taller, and it is somewhat earlier in showing ear.

Egyptian Barley.

This old variety is a two-rowed form, and in that respect is to be classed with Standwell, but it has a well-known peculiarity in that many of the plants bear ears which are more or less branched (Fig. 6, *c*). In extreme cases the branching may give rise to tufted ears. The specimen used as pollen parent was only slightly branched. The foliage of Egyptian and Standwell is of the same dark green, that of the former being slightly but appreciably less strong than that of the latter. Standwell is slightly earlier in showing ear.

Bere.

The ear (Fig. 5, *c*), actually six-rowed, has the appearance of being four-rowed. It is large, drooping, and extremely strongly awned. Clear

rose-purple lines beautify the exterior of the outer pales (flowering-glumes). Bere shoots into ear, flowers, and ripens considerably earlier than any of the barleys above described.

Only a few crosses were attempted, and the successful ones yielded the following grains: 4 as the result of the cross with Stranger, 3 with Zero, 1 with Egyptian, and 1 with Bere.

The grains were sown in the open. Two of the Stranger cross and two of the Zero cross died off, and the Bere cross was attacked at the root by some pest and was seriously checked. The remaining plants having plenty of room, the tillering in most cases was remarkably free. The hybrid nature of the plants was unmistakable in all cases.

Opportunity was missed of noticing the relative earliness of flowering of the hybrids. Their ears were more or less intermediate in character between the parental forms. The ears of the cross with Zero bore very little resemblance to those of the cross with Stranger (Fig. 4, *b*), the former having a marked leaning to the six-rowed type, while the latter had an equally marked leaning to the two-rowed type.

The number of the grains in individual ears have not been noted, but the following enumeration of grains taken from the single hybrid plants will serve to show their productiveness.

	Stems	Good ears	Tallest	Grains in ears	Average in each ear
Standwell × Stranger ...	54	39	4 ft.	673 in 30	22·4
,, ,, ...	33	14	4 ft.	287 ,, 12	23·9
,, Zero	32	21	3 ft. 9 in.	451 ,, 18	25
,, Egyptian	36	36	3 ft. 8 in.	820 ,, 29	28·2

The figures for the cross with Bere have not been put down, normal development having been checked. Four or five good ears of it were saved, and 82 grains secured for sowing.

The seed of all four hybrids was sown in lines in 1905. The first feature of interest noted was the evidence of the impression of the early character of Bere on its hybrid progeny. When the ears of Bere were almost clear of the sheaths, and those of Standwell were still quite enclosed, several of the hybrids showed ears projecting considerably from the sheaths. It soon became obvious that there were three marked types in the lines, those resembling either Bere or Standwell, and the hybrid forms. The early character of Bere was seen to be impressed

on a certain number of all three types, and it was interesting to see the plots at the stage when the Bere and its early hybrid progeny were in the ascendant, their ears alone being visible and projecting a foot or more above all the other barleys, both hybrid and parent, in the plots.

Bere was the first of the series to flower. It was followed next day by one of the progeny having marked hybrid characters. These were quickly followed by others which showed a strong leaning to either one or the other grandparent.

At about the time this stage was reached it was found that when 15 of the plants had their ears clear of the sheaths, 51 of them were showing various degrees of retardation, from the latest with the tops of the awns projecting only half an inch beyond the sheaths, to conditions approaching release of the ears. Although the line of demarcation between earlies and lates was not very strong, it was impossible to help thinking that the early forms might prove to be recessive and so be readily fixed.

It may be mentioned that the plants of the Zero cross were somewhat later than those either of the Stranger or the Egyptian cross. Earlier and later forms appeared in both the Zero and the Stranger crosses, whereas the Egyptian cross was very uniform in respect of its flowering period.

Standwell × Bere.

At harvest it was found that the plants of this cross could be divided into two series, two-rowed and six-rowed. The aggregate of the six-rowed, including the hybrid forms, was 55, and of the two-rowed 14, an approximation to the Mendelian ratio which seemed to warrant the assumption that the two-rowed character in this cross is recessive.

Two or three plants were found to be indistinguishable from Bere, even to the presence of the rose-purple lines on the pales. On the other hand, several bore a very great resemblance to Standwell, the only difference being that the grain of the former was not so plump as that of the latter. A number of the hybrid forms could perhaps be classed as identical with the hybrid parent (Fig. 5, *b*), a greater or less number of the usually sterile florets being fertile, but with small grains; and these forms were connected with the others closely resembling Bere or Standwell by many stages of variation. For instance, all stages seemed to be represented between the extremely strongly-awned pales and fully-developed grains corresponding to the extra (lateral) ones in Bere, through samples with weaker-awned pales and smaller grains in that

position, to types like Standwell in which both grains and awned pales were absent there.

Examples occurred in which the grains of all the six rows were fully developed, but the lateral ones were without awns. The awns present in the central grains in such cases varied in strength, being sometimes as strong as those of Bere.

Plants with ears of a remarkably long, open or lax character occurred in both two-rowed and six-rowed types.

Standwell × Stranger.

The plants of this cross were easily separated into two series, two-rowed and six-rowed. Of the former there were 165 plants, and of the latter 52. It seems quite certain that these figures satisfy Mendelian expectations, and that the six-rowed character, as has already been shown by other observers[1], is here the recessive one.

A very remarkable similarity of form existed amongst the six-rowed plants, and they differed from Stranger in being somewhat shorter in the ear. A few plants, however, had ears of the same size. It is possible that exact repeats of Standwell may have occurred, but such were not noticed.

The two-rowed plants were also much alike among themselves, and they differed from Standwell in the ear being somewhat longer and narrower. A further difference lay in their possessing or retaining very short awns on the pales of the lateral florets, whereas in Standwell these have disappeared and left the pales truncate at the apex.

One or two plants showed the hybrid characters more strongly in possessing lateral grains with awned pales, but both more or less poorly developed. On the other hand, examples with truncate pales occurred in a few of the hybrid forms. Nine or ten plants were found with particularly lax ears.

Standwell × Egyptian.

Time did not permit of a full and careful inspection of the plants of this cross. Less than half of the material was looked over. In the 251 plants examined, 208 were set down as normally two-rowed, and 43 as more or less of the Egyptian type.

It is highly probable that many having the Egyptian character

[1] See Biffen, "The Inheritance of Sterility in the Barleys," *Journ. of Agric. Science*, Vol. I. Part II.

inconspicuously developed would be placed in the larger bundle. In this connexion it is to be remembered that unbranched plants occur in plenty in fields of genuine Egyptian. Further, it is to be noted that, in selecting, both branched and unbranched ears were found on one and the same plant.

The original plant was, as is already mentioned, only slightly branched. Many of the present lot were more branched than it was, but comparatively few were very much branched.

The plants of the normal two-rowed lot were like Standwell, but there was some variation in the form of the ear, some of the ears being much longer and opener than those of Standwell.

Standwell × Zero.

Opportunity was missed of examining the plants of this cross after harvest. This is to be regretted, because it would have been interesting to compare them with the plants of the Stranger cross.

The writer has to acknowledge assistance from the Carnegie Trust for the Universities of Scotland, during the last two years of his experimental work.

Fig 1.—Ear of Goldfinder × Black Tartarian Oat.

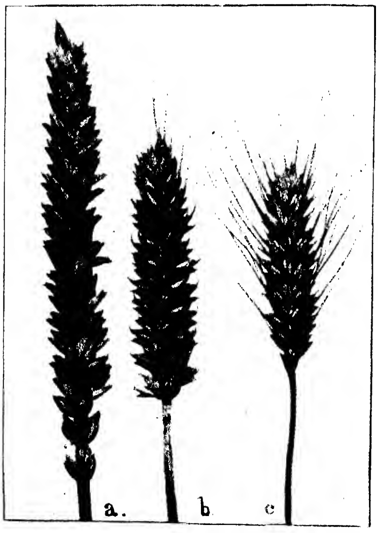


Fig. 2.—Ears of *a*, Red King; *b*, Hybrid; *c*, Rood Koren Wheat. Reduced.

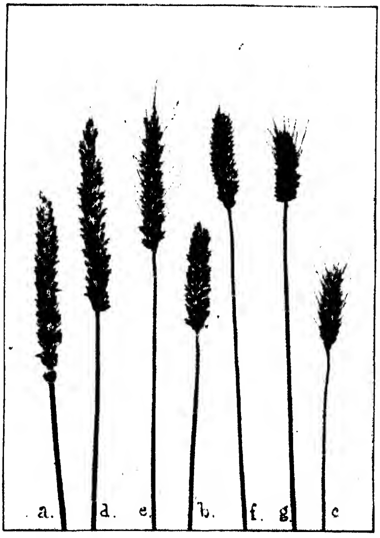


Fig. 3.—Ears of *a*, Red King; *b*, Hybrid; *c*, Rood Koren; *d*, *e*, *f*, *g*, four types derived from the hybrid.

Fig. 4.—*a*, Standwell; *b*, Hybrid; *c*, Stranger Barley. Natural Size.

Fig. 5.—*a*, Standwell; *b*, Hybrid; *c*, Bere. Natural Size.

Fig. 6.—*a*, Standwell; *b*, Hybrid; *c*, Egyptian. Natural Size.

THE HEAT VALUE OF MILK AS A TEST OF ITS QUALITY[1].

BY JOHN MALCOLM, M.D., AND A. A. HALL, M.A., M.D.

(*From the Physiological Laboratory, University of Edinburgh.*)

AN indirect method of estimating the heat value of food stuffs, such as milk, is to determine the amounts of fat, sugar, and proteid chemically, and to multiply by the average caloric value of each; but since the amounts of these substances themselves indicate the quality of the milk their calculated caloric value need not be employed as a test of its quality.

On the other hand, a direct estimation of the caloric value by combustion in an accurate calorimeter might be expected to give an exact gauge of the quality of the milk, and this at one operation occupying a comparatively short time.

The following experiments were undertaken in order to determine how far this direct calorimetry might be depended upon as a test of the adulteration of a sample of milk, when used alone or in conjunction with other data.

Methods. The instrument used in our estimations was a bomb calorimeter (Berthelot-Mahler). The interior is enamelled, except the inside of the lid, which is lined with platinum; the inlet tube for the oxygen acts also as the conductor for the electric current which ignites the substance.

The combustions were performed in oxygen at 23 atmospheres pressure. The "water equivalent" of the bomb, mixer, calorimeter vessel and thermometer (= 323 c.c.) was estimated by the method advised by Stohmann[2], and each 1° C. rise of temperature of the water

[1] The greater part of the work summarised in this paper was done by Dr Hall and presented by him for the M.D. degree as a thesis, for which he was awarded a gold medal.

[2] *Journ. für prakt. Chemie*, 39, S. 524, where much valuable information regarding the bomb calorimeter may be obtained.

placed in the calorimeter represented 2500 calories. The thermometer was graduated in hundredths of a degree C. and with a magnifying-glass could be read to one five-hundredth of a degree.

The correction for radiation (+) was calculated from the observed rate of rise and subsequent fall of temperature; owing to the rapidity of the combustion it seldom rose over ·02°.

A further (−) correction was made for the iron wire oxidized in the ignition and for the union of water with the acids formed by oxidation of the sulphur, nitrogen, phosphorus, etc. At first Kellner's method was followed in preparing the milk for combustion, *i.e.* the milk was dropped on to a known weight of cellulose, and dried. The following is an example of the results obtained:

Cellulose block (0·7286 grm.) saturated with 2·5 c.c. milk, dried at 50° C.

Water 2500 c.c.: rise of temperature 1·743° plus correction for radiation (0·016° C.) = 1·759°; giving $1{\cdot}759 \times 2500 = 4397$ calories: minus correction for iron wire used for ignition, and acids formed (22 calories) = 4375.

Average caloric value of cellulose used = 4182; ∴ deduct for cellulose $(0{\cdot}7286 \times 4182) = 3047$.

2·5 c.c. milk = 1328 calories = 531 calories per c.c.

The disadvantages of this method are very apparent. Only a small amount of milk can be experimented upon, for if the block be once saturated with milk and dried, it does not readily take up a second quantity owing to the dried fat forming a layer on the surface, and the correction for the cellulose is larger than the figure obtained for the milk itself.

After trying various other methods on Kellner's principle we fell back on simply drying the milk at 50° C.[1] in a shallow platinum vessel of about 12 c.c. capacity, which could be placed in the position in the bomb usually occupied by the carrier for the substance to be incinerated. The milk generally dried to a thin superficial scale with a deeper layer on the bottom of the dish, and the ignition wire (iron) was either placed so as to touch this, or a cotton thread, placed partly in the milk previous to drying, was made to hang over the wire and to act as the carrier of the igniting spark.

10 c.c. milk was taken each time, but the results are given for convenience as so much per c.c.

[1] The drying was not complete; we generally aimed at having 5 to 7 °/₀ moisture in the dried residue, as that amount is usual in estimations of the heat value of food stuffs.

As shown in the control experiments (Table I), and in the comparison of direct and indirect methods of estimating the caloric values (Table IV), the method gave accurate results.

I. *Comparison of Heat Values of the same and of different Samples of Milk.* (Table I.)

In this, as in all the other cases, samples of the milk were obtained from various dairies in Edinburgh, and "sweet milk" was asked for on each occasion. In doing controls of any one sample of milk dilutions with water were employed so as to test the accuracy of the method with development of different amounts of heat. (The form of bomb calorimeter used is said to be most accurate when the amount of heat involved is about 7000 cal.)

TABLE I.

	Calories found per c.c.	Calories expected per c.c.	Differences in calories per c.c.
Milk No. I.	676	—	
Same diluted with an equal amount of water...	337	338	1
Milk No. II.	753·5	—	—
Same diluted with an equal amount of water...	379·4	376·7	2·7
Milk No. III.	652·8	—	—
Same diluted with 10 °/₀ water	581·7	586·8	5·1
Milk No. IV.	877·4	—	—
Same diluted with 7½ °/₀ water	813·0	811·5	1·5
Milk No. V.	776·0	—	—
Same diluted with 8 °/₀ water	726·0	717·6	8·4
Milk No. VI.	650·0	—	—
Same diluted with 4 °/₀ water	623·6	624	0·4
Same diluted with 8 °/₀ water	585·4	598	12·6
Milk No. VII.	868	—	—
Same diluted with 5 °/₀ water	829·4	824·6	4·8
Same diluted with 10 °/₀ water	784·7	796·2	6·5

These results are given in the order in which the specimens of milk were examined and include all that were done at that time (with two exceptions in which some unconsumed carbon was found, in which, therefore, the combustion had been incomplete).

The estimations were done by one of us (A. A. H.) who had very little previous experience of the calorimeter; they show, therefore, that accuracy in this kind of work can be readily acquired.

The table also exhibits the efficiency of the calorimeter over a wide range of heat developments (from 3370 cal. in Milk I (diluted) to 8246 cal. in Milk VII). The highest difference between observed and expected calories was 12 cal. per c.c. in VI (diluted with 8 °/₀ water). Taking one grm. of fat as having a caloric value of 9318 cal. (Rubner), this number of calories represents ·0014 grm. fat, so that the maximum error in these estimations is a small one, compared with the error in fat estimation by weighing.

II. *Relationship of Fat percentage to Caloric Value.* (Table II.)

Here the caloric value was determined as before, single estimations only being done. The fat was estimated by Adam's method (5 c.c. milk dropped on to fat-free paper, extracted with ether in a Soxhlet apparatus, and the extract dried and weighed); controls were done in each case.

The following results were obtained in milks from various dairies: they are arranged in order of fat percentage.

TABLE II.

No. of Milk	Percentage of fat	Total calories per c.c. by calorimeter	Calories per c.c. due to non-fatty substances
VIII	5·4	888	383
IX	5·4	868	363
X	5·3	829	336
XI	5·1	848	373
XII	4·9	784	328
XIII	4·7	776	338
XIV	4·3	726	326
XV	4·0	690	318
XVI	3·9	663	300
XVII	3·8	682	328
XVIII	3·3	683	376
XIX	3·1	675	386
XX	3·0	628	349
XXI	2·9	601	331
XXII	2·8	607	346
XXIII	2·7	652	401
XXIV	2·6	566	324
XXV	2·5	570	338
XXVI	2·1	540	345

These figures show that there is, as one would expect, a general relationship, but no constant ratio. This results from the fact already observed by Vieth and others that milks of high fat percentage do not have a correspondingly high sugar and proteid percentage. In other words, the "solids-not-fat" is a fairly constant figure in milks, and this is also brought out in Table II, last column. We have here deducted the calories due to fat from the total calories (assuming 1 grm. fat = 9318 cal.). The figures are fairly even throughout the series, showing that the lower fat percentages were probably skimmed or separated milks, while in the milks with high fat percentages the increase in caloric value was mostly due to the increased fat. (In Milk XXIII there has probably been some mistake, either in fat estimation or in calorimetry.)

The heat value which corresponds to a milk containing 3 per cent. of fat is over 600 calories, and probably 650 would make a fair figure for a legal standard.

If that were the standard, a dishonest milk-seller would find it difficult to dilute a rich milk and yet escape detection: suppose he diluted Milk VIII so as to reduce the fat percentage from 5·4 °/₀ to 3 °/₀ he might still escape under the present legal standard, but the caloric value would be

$$\left(\frac{3}{5{\cdot}4} \times 888\right) = 493 \text{ cal.},$$

sufficiently below the standard caloric value for a clear verdict.

III. *Relationship of Total Solids to Caloric Value.* (Table III.)

This was next investigated on similar lines.

The solids of 10 c.c. milk were weighed after drying to constant weight.

The average given by these figures, 12·4 °/₀ solids, equivalent to 720 cal. per c.c. or 5800 cal. per gramme, is higher than would be given by a milk which just satisfies the present legal standard. That standard requires 3 °/₀ fat, and 8·5 °/₀ solids not fat: by deducting 0·7 for ash and dividing the remainder in the proportion given by Vieth for proteid and sugar in cow's milk, we obtain—sugar 4·6, proteid 3·2, and this gives a theoretical value for the caloric value of total solids of about 5650 per gramme. The last column gives the result of applying this to the figures obtained for total solids, and it will be noticed at once that while the rich milks give more calories than one would

TABLE III.

No. of Milk	Solids %	Calories per c.c.	Calories expected if 1 grm. solids = 5650 cal.
XXVII	14·4	868	813
XXVIII	14·2	896	802
XXIX	13·0	812	734
XXX	12·0	682	678
XXXI	12·0	650	678
XXXII	11·8	675	666
XXXIII	11·2	607	632
XXXIV	10·9	572	615
Average	12·4	720	

expect, owing to the increased solids being due to fat, the poor milks show a much lower heat value than expected from the total solids, *e.g.* the solids in XXXIII and XXXIV must have been very poor in fat.

We consider this an important means of detecting removal of fat from milk, and the estimation of the total solids can be readily carried out along with the estimation of heat value.

IV. *Complete Analysis compared with Caloric Value.* (Table IV.)

The methods employed here were the usual ones. *Proteid* was estimated by determining the amount of nitrogen in the precipitate obtained with Almén's solution, and multiplying the result by 6·37; *lactose*, by titration with Fehling's solution after removal of the proteid by acetic acid and subsequent boiling; *fat and caloric value* as before; the *ash* by slow incineration of the total solids.

None of these figures have been brought into the other tables, but a consideration of them confirms the previous work. Thus the accuracy of the calorimeter is shown by the correspondence between the last two columns where the caloric value is given by the calorimeter and also by calculation, using the factors 9318 for fat (Rubner), 5860 for milk proteids [average of 5855 (Danilewsky), and 5867 (Stohmann)], and 3950 for lactose (Rubner). The absence of a constant ratio between fat percentage and caloric value is evident. The estimation of the calories expected from the total solids again shows that the milks with

TABLE IV.

No. of Milk	°/o Total solids	°/o Lactose	°/o Proteid	°/o Fat	°/o Ash	Calories estimated (per c.c.)	Calories calculated (per c.c.)
XXXV	10·83	4·25	3·08	2·66	·73	572·0	590
XXXVI	14·40	4·82	3·40	5·40	·74	888·5	892
XXXVII	10·82	4·18	3·26	2·50	·76	570·8	589
XXXVIII	11·76	4·48	3·34	3·10	·77	669·4	661
XXXIX	11·08	4·23	3·08	2·88	·76	607·7	614
XL	11·96	4·72	3·36	3·01	·73	656·7	660
XLI	9·79	3·75	3·13	2·11	·72	531·5	528
XLII	12·01	4·21	3·06	3·82	·74	682·7	701
XLIII	13·10	3·80	3·49	5·10	·75	812·6	830

low solids were poor in fat. For example, XXXVII with 10·82 total solids ought to have yielded over 600 calories, whereas only 570 were obtained, and analysis also bears this out (2·5 °/o fat).

Summary. The suggestion is made that the direct determination of the caloric value of milk would be an important aid to analysis in the detection of removal of fat from milk or the dilution of milk with water. The operation requires little chemical knowledge, is easily learned, is very accurate and occupies a relatively short time (forty minutes or less after the milk has been dried). After the initial expense of the calorimeter (about £35) the running expenses are light (about sixpence per combustion).

The legal definition of milk might be improved by the adoption of a minimal caloric value per c.c. or per gramme of total solids.

We consider our estimations too few for determining what that standard should be, but in order to correspond with the present legal standard it should not be below 650 cal. per c.c. or 5650 cal. per gramme solids.

The expenses of this research were borne by the Moray Fund for the Endowment of Research in Edinburgh University.

ON THE EVOLUTION OF GAS DURING CHURNING.

By R. D. Watt, M.A., B.Sc., *Carnegie Research Scholar, Rothamsted Experiment Station.*

It is well known that at the beginning of the churning process a considerable volume of gas is evolved, necessitating some method of ventilation of the churn. In old-fashioned churns the gas was allowed to escape by simply withdrawing the cork or plug, while all modern churns have a special arrangement for the purpose. The gas set free has been stated to be carbon-dioxide, but hitherto no definite proof of its source or estimation of its amount has been recorded. It cannot very well be that the gas is formed by any chemical reaction at the time the churning process is going on. It seems more probable that it is produced by bacterial action during the ripening of the cream, held in a state of super-saturation by the liquid and released by the shaking in the churn in the presence of air. This view would account for the fact that it is only during the first hundred or so revolutions of the churn that any quantity of gas is evolved. It was with the object of demonstrating the correctness or otherwise of this latter theory that the following experiments were carried out.

The apparatus used for determining the amount of carbon-dioxide in each case was a simple modification of that for determining small quantities of carbonates in soils described by Mr Arthur Amos, B.A., in the first volume[1] of this journal. The condenser was found unnecessary, and, as no acid has to be added, the thistle-funnel was also dispensed with. The glass tube leading from the first Reiset apparatus (A) was made to extend almost to the bottom of the Jena flask (B), so that air free from carbon-dioxide could be bubbled through the cream, thus assisting in the expulsion of the gas.

As a preliminary to the determinations with cream the amount of

[1] Vol. i. Part 3, p. 322.

CO_2 present in three samples of milk freshly drawn from the same cow on three successive mornings was ascertained. To ensure accuracy the Jena flask and the rubber tubing in connexion with it were detached, the latter being secured by clips. This piece of apparatus was weighed and a quantity of milk (about 300 c.c.) was milked directly into the flask, which was immediately corked up. The whole was then weighed again, the difference giving the weight of the milk. The connexions were made with the two Reiset towers and the pump started, the clip nearest the pump being removed first, so that the carbon-dioxide liberated from the milk during transit was all absorbed by the sodium hydrate in the second Reiset (C). The milk was then gradually heated to the boiling-point and the titration carried out in the usual way.

The amount of carbon-dioxide found was as follows:

1st morning	... ...	78·18 c.c. per litre
2nd ,,	... ...	79·22 ,, ,,
3rd ,,	... ...	77·00 ,, ,,

One quart of cream extracted by the separator from the milk of a herd of Jersey cows was obtained on four separate occasions.

(1) 200 c.c. of this were tested for CO_2 by the above process soon after separation.

The remainder was allowed to stand for 3 to 3½ days at a temperature of from 15° to 17° C., at the end of which time it was in the usual condition for churning.

(2) 200 c.c. of this were taken and the amount of carbon-dioxide present ascertained.

Other 200 c.c. were placed in a large bottle with about 100 c.c. of water, the resulting temperature being about 17° C. (62° F.). The bottle was shaken vigorously in imitation of churning, the evolved gas being allowed to escape by withdrawing the cork a few times.

(3) The amount of CO_2 remaining in this partially churned cream was also determined, the results being shown in the following table:

No. of determination	No. of c.c. carbon-dioxide found per litre of cream		
	(1) after separation	(2) after ripening	(3) after partial churning
1	51·35	233·25	80·35
2	37·95	283·50	51·35
3	39·05	246·50	54·70
4	27·90	142·85	45·75

At ordinary temperatures pure water, or such a dilute solution as forms the serum of cream, dissolves about an equal volume of CO_2 when saturated in contact with pure gas. On exposure to air, however, almost the whole of this gas will come out of solution in accordance with Dalton's law of partial pressures, the amount remaining dissolved being proportional to the percentage of carbon-dioxide in the gaseous mixture now in contact with the liquid. From such a saturated solution, however, the CO_2 only escapes very slowly unless the liquid be agitated; brisk shaking will cause it almost immediately to assume its new condition of equilibrium with the air. In the case under investigation we see that the cream after ripening is considerably supersaturated with carbon-dioxide, so that about 200 c.c. per litre of cream are instantaneously liberated by shaking, before the solution adjusts itself to the mixture of air and carbon-dioxide that is formed within the churn. In an ordinary small churn for churning about four gallons of cream the volume of carbon-dioxide set free would be about $3\frac{1}{2}$ litres, and this, together with the slight rise of temperature, seems quite sufficient to account for the need for ventilation.

As to the source of the carbon-dioxide, though the lactic acid bacteria which take part in the ripening of cream are sometimes considered to effect a simple change from milk-sugar into lactic acid, according to the equation

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3,$$

Fleischmann[1], Richmond[2], and others[3] have pointed out that oxidation to carbon-dioxide takes place as well. I am informed that cream, ripened with the anaerobic lactic acid organism which does not produce gas, requires no ventilation during churning.

The acidity of the cream before and after ripening in each case was estimated by titrating 50 c.c. with decinormal sodium hydrate, using phenolphthalein as indicator, with the following results:

No. of sample	No. of c.c. $\frac{N}{10}$ NaOH required to neutralise 50 c.c. cream			Increase in CO_2 per litre during ripening	Percentage of increased acidity due to CO_2
	Before ripening	After ripening	Increase		
1	7·5	38·2	31·7	181·90 c.c.	12·8 %
2	8·0	41·5	33·5	245·55 ,,	16·4 ,,
3	7·0	37·8	30·8	207·45 ,,	15·0 ,,
4	7·9	37·8	29·9	112·95 ,,	8·5 ,,

[1] Fleischmann's *Book of the Dairy*, English translation, pp. 24, 26, 99.

[2] Richmond's *Dairy Chemistry*, pp. 18, 226.

[3] *E.g.* Swithinbank and Newman's *Dairy Bacteriology*, p. 154.

This would indicate that the amount of carbon-dioxide compared with the lactic acid formed is large; but it must be remembered that a very considerable proportion of the latter is neutralised by alkaline phosphates, etc.

Conclusions.

A considerable volume of carbon-dioxide is produced by bacteria during the ripening of cream and held by it in a state of supersaturation.

The agitation in the churn very soon brings about a new condition of equilibrium in accordance with Dalton's law of partial pressures, a large percentage of the carbon-dioxide being liberated. This, together with the slight rise in temperature, is sufficient to account for the necessity to ventilate the churn at intervals during the first few minutes of the process.

The amount of CO_2 produced bears no very constant relation to the lactic acid or to the total acidity.

I have to acknowledge my indebtedness to Mr A. D. Hall, M.A., at whose suggestion and under whose supervision the investigation was carried out, and also to the Lawes Agricultural Trust for the use of the laboratory and apparatus.

THE PRESERVATION OF EGGS BY WATER GLASS AND THE COMPOSITION OF THE PRESERVED EGGS.

By JAMES HENDRICK, B.Sc.,
University of Aberdeen.

One of the most popular and commonly used methods of preserving eggs is by means of water glass. Though this method was introduced only comparatively recently it has largely superseded older methods, and also appears to have led to much more frequent preservation of eggs on the small scale in households and by small traders. The method is simple and effective. The eggs are obtained when they are plentiful and cheap in spring and preserved for use during the winter months. It is therefore necessary to keep them for about six months. In the experiments described below some were left in a solution of water glass as long as four years.

Water glass is a silicate of soda, and is used for a variety of purposes. It is prepared as a thick syrup for use as an egg preservative. In Table I. analyses of two samples are given, and also an analysis of sample 2 diluted with water, for use as an egg preservative. The solutions used in most of my experiments were of about the strength shown in this analysis. The analyses show that silicate of soda used for egg preservation does not contain quite sufficient soda to form the acid metasilicate, $NaHSiO_3$. The solution given by the water glass is strongly alkaline in reaction.

Table I.

Composition of Water Glass.

	1.	2.	
		Original	Solution of same used for preserving eggs
Silica	36·87 per cent.	37·91 per cent.	2·76 per cent.
Soda	16·01 ,, ,,	16·48 ,, ,,	1·20 ,, ,,
Potash	0·14 ,, ,,	0·14 ,, ,,	0·01 ,, ,,

A little Carbonate was present in both samples.

Na_2SiO_3 contains Silica 49·18 per cent., Soda 50·82 per cent., Water —,
$NaHSiO_3$,, ,, 60·0 ,, ,, ,, 31·0 ,, ,, ,, 9·0 per cent.

My experiments were at first started only to find out whether eggs would remain for long periods, such as two or three years, in dilute solutions of water glass without undergoing decay or any other serious change of composition. In ordinary practice it is only necessary to preserve the eggs for about six months; my object was to find out whether the eggs would remain good for a much longer period.

Through the kindness of a retail grocer in Aberdeen, who annually preserves a large number of eggs, I was enabled to examine large numbers of eggs which had been preserved in water glass, and also to set aside annually for a number of years small experimental lots of eggs which were kept under the same conditions as those which were preserved for ordinary trade purposes. The eggs were not preserved under the very best conditions, as they were not put into the water glass day by day as they were laid, but were collected in the country and sent into town in large lots before preservation. Most of them therefore were two or three days old before being placed in the preservative. It is therefore the more remarkable that not a single egg in the small experimental lots was ever found to be bad or even tainted. These eggs were of course carefully selected and packed. As was to be expected in the ordinary trade lots, which were preserved in large tubs, a few unsaleable eggs were always found. These were generally chipped or cracked eggs. They had been injured either before being preserved, or during the packing in the preserving tubs. It was very seldom that a really bad, decomposed egg was found, and there is every reason to believe that when any such were found their presence was due to the inclusion of old and tainted eggs among those originally sent in from the country.

The eggs were examined before being put in the preservative, but it is not possible to detect all the slightly decomposed eggs by a mere cursory examination. A note was kept of the total number of unsaleable eggs in certain consignments which were preserved. For instance, in 1905 out of 384 dozen preserved between April and June, and sold between October and December, five dozen, or 1·3 per cent., were bad. The great majority of these were broken or cracked eggs.

Eggs which are preserved in water glass have a nice appearance, as the shells are very clean and fresh looking after the water glass is wiped off them. Even those which had been several years in water glass had a fine fresh appearance. Another advantage of preservation in water glass over certain other methods is that the contents of the egg do not shrink owing to evaporation. The eggs therefore do not rattle when

shaken, no matter how old they are. The cost of preservation is very small.

It was found that eggs which had been kept in water glass for a few months could hardly be distinguished in appearance, flavour and smell, either raw or cooked, from what are called "fresh eggs," that is fresh eggs in the commercial sense, which are eggs which should be free from decomposition or taint, but which may be several days old. A really fresh egg, only a few hours laid, is easily distinguished in flavour and appearance when cooked from the "fresh egg" or preserved egg, and is known as a "new-laid" egg. The eggs which had been preserved in water glass for about six months tasted and smelt like well-kept eggs a few days old. As the eggs in question were a few days old when they went into the water glass, they were not appreciably changed to my eye and palate by a few months' stay in water glass.

As the eggs get older however a distinct change is found which can be appreciated both by the eye and palate. Eggs which have been three or four years in water glass are easily recognised. The white becomes pink in colour and very liquid. The egg acquires a slightly peculiar taste which to my palate suggested soda. At the same time even when four years old the eggs had no unpleasant taste or smell, and the white coagulated in the usual manner in cooking. Though there was a slight characteristic odour when the eggs were cooked, it was not a stale or bad odour and did not suggest sulphuretted hydrogen. The changes in the preserved eggs take place very gradually. At one year old they are hardly noticeable; at two years they are distinct, but not so distinct as at three or four years old.

The above observations, which have no doubt been made by many others, satisfied me that eggs could be preserved in water glass for long periods without decomposing or undergoing any other serious change. As the experiments progressed, however, it was decided to enlarge their scope by determining whether any distinct changes take place in the composition of eggs when they are kept in water glass, and especially whether the soda and silica of the water glass penetrate into the egg to any great extent.

There are comparatively few analyses of eggs, and especially of the ash of eggs, on record. The eggs used all through these experiments were those of the ordinary barndoor fowl. The eggs of ducks, turkeys and other less common fowls were excluded. König (*Menschlichen Nährungs u. Genussmittel*) records a few analyses of eggs, but does not give many ash analyses. Other writers on foods generally quote

König. There are also a few recorded American analyses of eggs, but these do not give ash analyses. I have not succeeded in finding any recorded analyses of preserved eggs, nor am I acquainted with sufficient ash analyses to show what variations may be expected in the different ash constituents of ordinary commercial eggs.

In Tables II. and III. analyses of a number of fresh eggs and of eggs of different ages preserved in water glass are recorded. Owing to an unfortunate accident a portion of the laboratory records on this subject were destroyed, and therefore the analyses of a number of samples are wanting and some of those given in the tables are fragmentary. Each analysis was made on the mixed contents of at least three eggs, and all analyses were made in duplicate.

TABLE II.

Composition of Fresh Eggs.

	Average from König per cent.	1. 3 Eggs per cent.	2. 3 Eggs per cent.	3. 4 Eggs per cent.
Moisture	73·67	73·18	72·70	74·44
Nitrogen	2·01	2·11	—	—
Fat	12·11	10·40	—	—
Ash	1·12	1·02	1·36*	1·06
Ash Soluble in water	—	—	0·55	0·56
Potash	0·159	0·120	0·156	0·139
Soda	0·200	0·194	0·173	(?)
Silica	0·003	0·010	0·021	0·031

* Contained a little carbon.

In Table II. the average analysis of the eggs of barndoor fowls as recorded in König's *Menschlichen Nährungs u. Genussmittel* is given, and then partial analyses made in my laboratory of three samples. It will be seen that the analyses are in general agreement. As the figure for silica is an important one, it was determined carefully in large samples of 40 to 50 gms. of egg.

TABLE III.

Composition of Preserved Eggs.

Preserved / Analysed	1902 / 1904 per cent.	1902 / 1905 per cent.	1903 / 1904 per cent.	1903 / 1905 per cent.	1904 May / 1904 Octr. per cent.
Moisture	72·12	74·66	73·55	73·73	—
Nitrogen	2·17	2·10	2·01	2·07	—
Fat	11·19	9·42	10·70	10·41	—
Ash	0·92	1·00	0·93	1·02	—
Potash	0·075	0·069	0·101	0·073	0·143
Soda	0·296	0·343	0·215	0·311	0·200
Silica	0·023	0·019	0·022	0·039	—

In Table III. partial analyses are given of five samples of preserved eggs. These represent eggs six months, one year, two years, and three years old, and preserved in three different years.

On comparing Tables II. and III. it will be seen that the analyses of the fresh and of the preserved eggs, apart from the details of the ash analyses, are in close agreement. As already pointed out, in water glass the eggs do not dry up, and the tables show that the percentage of moisture in the preserved eggs is quite similar to that in the fresh eggs. There is also practically no change in the percentage of ash. It was anticipated that the ash of the preserved eggs would be greater than that of the fresh eggs, but this anticipation obtains no support from the figures. The ash of the preserved eggs contains no more silica than the ash of the fresh eggs. The oldest sample in Table III. contains less silica than some of the fresh eggs in Table II. We may conclude therefore that silica does not diffuse through the shell into the contents of the egg at all.

The soda slightly increases in the preserved eggs, and the increase appears to be according to the length of time the egg was in water glass. The whole increase in soda however is very slight, and is not comparable with the strength of the soda solution in which the eggs were immersed. Table I. shows that the eggs were immersed in a solution of water glass containing over 1 per cent. of soda. The soda in the eggs which were two or three years in the solution does not appear to have increased more than about 0·1 per cent. There are so few ash analyses of eggs that it cannot be concluded that the apparent increase in soda is not due, in part at least, to natural variations in different samples of eggs. It is remarkable that in Table III. as the soda increases the potash appears to diminish. This may be due to a slow interchange between the potash of the egg and the soda of the solution. But there is another possible explanation, namely, that eggs which are naturally high in soda are low in potash, or that in eggs as in plants, soda and potash are able to replace one another naturally to a certain extent. It is not possible to decide in the absence of extensive series of ash analyses of eggs whether the variation is due to interchange between soda and potash or to natural variations in eggs, but the evidence is so consistent as to make it highly probable that when preserved in water glass the soda of the egg slowly increases, and a very small diminution of the potash takes place. The slight alteration in the flavour of the egg and in the liquidness of the white may be due to the increase in soda.

The alkalinity of the contents of the eggs appeared to increase with the length of time they were in water glass, but the increase was small, and in a complicated substance like egg it was found difficult to measure it accurately.

The general conclusion to be drawn from Tables II. and III. is that there is practically no change in the composition of eggs even from lengthened immersion in water glass. Practically no silica and very little, if any, soda find their way into the eggs.

Analyses of the shells of a number of samples of eggs were also made chiefly in order to determine whether much silica was deposited in the shell. The results of these analyses are given in Table IV. In all cases the shells were those of eggs the contents of which had been removed for analysis (Tables II. and III.). The shells were not washed out in any way and therefore always had a little of the white adhering to them. They were allowed to stand in air in a dry, warm place till quite air-dry and brittle, but were not dried in an oven. The shells, including the membranes and any white sticking to them, were then ground and mixed. Each sample consisted of the shells of at least three eggs.

TABLE IV.

Composition of Shells.

Fresh Eggs in Preservative	From / To	Spring 1905 / Spring 1906	Spring 1903 / Summer 1905	Spring 1902 / Summer 1905
Moisture	3·45	2·95	3·12	2·85
Combustible Matter	5·13	4·32	8·60	8·47
Ash	91·42	92·72	88·28	88·68
Lime	49·05	49·16	48·15	48·15
Equal to Carbonate of Lime	87·59	87·78	85·98	83·98
Silica	0·57	1·64	1·95	2·32
Percentage of Lime in Ash	53·65	53·02	54·54	54·30

The considerable differences in organic matter in the different samples are probably largely due to different quantities of white adhering to different samples. If we omit the different quantities of organic matter adhering to the shells, the main difference between the different samples is in the amount of silica which they contain. In the fresh eggs this is about ½ per cent., and it increases according to the length of time the eggs have been in the solution. In the eggs which were three years in the solution the silica amounts to nearly 2½ per cent. It appears then that a slow deposition of silica takes place in the shell of the egg. The percentage of lime in the shells remains practically constant.

This deposition of silica in the shells probably blocks up the pores of the shells to some extent and renders them less permeable.

MICROBIOLOGIE AGRICOLE, pp. 439. E. KAYSER.

(J. B. BAILLIÈRE, Paris, 1905.)

DURING the last quarter of a century much light has been thrown by mycologists upon the relation of micro-organisms to the fertility of the soil. No concise and yet adequate account of their researches has hitherto been offered to the student of agriculture either in this country or on the continent. Conn's *Agricultural Bacteriology* fails to cover the ground, and the information in the translations of Alfred Fischer's *Vorlesungen über Bakterien*, and in the first edition of Lafar's *Handbuch der Technischen Mykologie* is scattered and insufficient.

M. Kayser, Microbiologist to the National Institute for Agriculture of France, devotes half of his book to an account of the mycology of the soil, and if one includes the chapters on 'ensilage' and 'milk' two-thirds of the work is of direct interest to the student of agriculture.

The book is divided into three parts: (1) an introduction dealing with bacteria in general; (2) the relation of micro-organisms to the fertility of the soil; and (3) microbiology applied to the transformation of (*a*) vegetable and (*b*) animal products.

The second part discusses the distribution of micro-organisms in the cultivated soil, their rôle in the fermentation of farm-yard manure, nitrification, denitrification, nitrogen fixation, the purification of sewage and industrial waste waters and finally the 'sulphur' and 'iron' bacteria. The whole of this account is excellent, and does not neglect the results of the most recent work. Dealing with farm-yard manure the author clearly distinguishes the aerobic from the anaerobic changes, and gives special notice to the specific organisms bringing about the fermentations of cellulose and urea. He assumes, however, both in this connexion and again when dealing with 'the septic tank' that methane is only produced from cellulose or similar bodies, whereas it was shown long ago that the gas is also formed during the putrefaction of proteids.

In the section on 'nitrification' a detailed account is given of the

method employed by Winogradsky for isolating the nitrous and nitric organisms and of their behaviour in artificial culture. The recent work of Boullanger and Massol, which has gone a good way towards clearing up the symbiotic relations of the two organisms under natural conditions, is also fully discussed. On page 99 the figures quoted from Wolff showing the amounts of nitric nitrogen in various soils to a depth of 20 centimetres are surely exceedingly high. A reference is made to the thermo-chemistry of nitrification, and the two following equations given:

$$2NH_3 + 6O = 3H_2O + N_2O_3 + 157 \text{ cal.}$$

$$N_2O_3 + 2O = N_2O_5 + 37 \text{ cal.}$$

One would like to know which 'calorie' is here employed. The author does not attempt to calculate out the amount of energy available from nitrous oxidation for decomposing carbonic acid, although Winogradsky's figures, showing that about 36 parts of ammoniacal nitrogen are oxidised for every part of carbon fixed, are quoted. It is stated that '*nitro-somonas*' forms a surface film on solutions in pure culture, but there is no reference to this property in Winogradsky's recent account of nitrification for the second edition of Lafar's *Handbuch.* We are also informed that the oxidation of ammonia to nitrous acid is due to an 'oxidase'; this may well be so, but it has not yet been proved.

Dealing with 'nitrogen fixation' three conditions are distinguished, viz. fixation by free-living soil organisms—*Clostridium Pasteurianum* and *Azotobacter*, fixation by symbiosis of algae and bacteria, and by symbiosis of bacteria with leguminous and certain other green plants. In connexion with fixation of nitrogen by Beijerinck's '*Azotobacter*' the author says that the Dutch mycologist considers many species of bacteria to fix free nitrogen and divides them into oligo-, macro- (*sic*) and polynitrophiles according to their activity for fixation. A reference to Beijerinck's paper shows this as a misquotation, his classification being into oligo- micro-, and polynitrophiles according to their ability to grow on media containing little or much combined nitrogen. There is a good account of 'nitrogen fixation' by leguminous plants, although some of Hiltner's recent speculative but stimulating notions are not clearly separated from his facts.

In the third part a description is given of the most important moulds, yeasts, and bacteria concerned in the industries closely allied to agriculture, and then a short account of the microbiology of the industries themselves, viz. brewing, distilling, vinification, cider-making, production of vinegar, starch manufacture, baking, retting of flax,

preparation of tobacco, tanning, and especially the preparation of ensilage and milk products.

In the introduction the author has something to say about enzymes, and again in the chapter on ensilage, but he calls them by the confusing name of 'diastases' after the custom of his fellow-countrymen.

There is a classification of bacteria, very simple, antiquated and inaccurate, in which 'bacillus' is distinguished from 'bacterium' as a longer and narrower rod; one notes also a contradiction, for after characterising the genus 'Sarcina' as 'endospore bearing' and including it in Coccaceae, the statement is made two pages later that the Coccaceae never have spores.

The book is very well illustrated from microphotographs and drawings, although the magnification is in many cases not given. The index might have been more extensive and there is no bibliography, but that was hardly to be expected in a work of the size.

If only for the sake of the part on 'the microbiology of the soil' this little book can be strongly recommended to the agricultural student who reads French, and a translation would be a useful addition to the English text-books of agricultural science.

S. F. ASHBY.

VOLUME II APRIL, 1907 PART II

STUDIES IN THE INHERITANCE OF DISEASE-RESISTANCE.

BY R. H. BIFFEN, M.A.,
Agricultural Department, Cambridge University.

IN a previous paper dealing with the inheritance of certain characteristics of wheat, evidence was brought forward to show that it was probable that liability and immunity to the attacks of yellow rust (*Puccinia glumarum*), were a pair of characters in the Mendelian sense of the word[1]. The evidence was as follows: Rivet wheat, a variety which is only slightly attacked by this parasite, was crossed with an extremely susceptible variety Red King. The latter is a hybrid descended from Michigan Bronze, which in turn is extremely susceptible to yellow rust. The resulting hybrids were as susceptible to the attacks of yellow rust as the parent Red King, whilst the generation raised from them consisted of individuals which were either slightly or excessively susceptible to the disease. There was a general agreement between the extent of the disease in these two classes and in the two parents, and further the proportions of badly to slightly attacked were in the ratio of 3 : 1. In the following generation the relatively immune individuals bred true to this character, though not necessarily to other characters as well, but the susceptible types taken as a class produced either all susceptible offspring or a mixture of susceptible and relatively immune—the result expected if these characters are Mendelian. Unfortunately the statistics for this last generation were not altogether satisfactory, partly owing to unfavourable conditions at the sowing time and partly owing to the excessive mortality of the diseased individuals in the preceding generation which limited the amount of grain harvested for this trial.

While the experiments were in progress a further series was commenced in order to obtain data on a more extensive scale and

[1] Biffen, *Journ. Agric. Sci.* Vol. I. p. 40.

also to determine whether immunity to the attacks of other fungi could be traced in a similar fashion.

A survey of the commoner plant diseases seemed to indicate that the Uredineae were the most suitable for this purpose and that the yellow rust offered unusual advantages, owing to the fact that one could count with no small degree of certainty on there being sufficient rust each season to thoroughly expose all plants to the chance of infection without having recourse to artificial inoculation. In this district a year never passes without there being a more or less severe epidemic of yellow rust, so that under ordinary circumstances there appeared to be no likelihood of failure through lack of suitable external conditions.

The black rust (*Puccinia graminis*) was also included in these experiments, though it was not expected to be so satisfactory for an investigation of this kind, owing to the fact that its appearance on the plots at the University Farm is generally late in the season. It rarely occurs before the middle of June and by that time the foliage of the wheat is often partly killed by the attacks of yellow rust. In some seasons no signs of it are to be met with. On the other hand the importance of the disease from an economic point of view had to be considered, and as a plentiful supply of infecting material could generally be obtained from its alternate host plant, the barberry, growing in a neighbouring hedgerow it was resolved to make the attempt and resort to artificial infection should the natural fail.

The investigation of the inheritance of liability to the attacks of a totally different group of fungi seemed essential, and as it was desired to work as far as possible with cereals the mildew *Erysiphe graminis* was chosen. Experiments with the biologic forms on both wheat and barley were planned but in the end only that on barley was carried to its conclusion.

The chief points of the life history of these parasites as far as they are concerned in this investigation may be briefly stated here[1].

The presence of *Puccinia glumarum* is indicated by the formation of numerous shortly elliptical pustules which break through the epidermis of the host plant—in this case wheat—and shed masses of spores known as uredospores. In the mass these have a characteristic deep cadmium yellow colour. These spores if applied to the surface of a variety of wheat susceptible of infection germinate and push hyphae

[1] For a complete account and figures, reference may be made to Eriksson, *Die Getreide-roste*, p. 141.

through the stomata, the mycelium then penetrates the intercellular spaces and drives haustoria through the walls of adjacent cells in order to obtain nutriment. If the conditions are favourable for development the mycelium will produce a fresh crop of uredospores in about ten days. Under these circumstances the spread of the disease is often exceedingly rapid and it is no unusual occurrence to see a field which is apparently rust free coloured yellow with the rust a week later. The period at which the uredospores are first noticed here is very variable, ranging from the beginning of March until the middle of May. Their formation continues until the foliage begins to die, generally about the middle or end of July, and then the mycelium produces a second spore form, the teleutospore, in small, flat sori. Whether a third spore form, the aecidiospore, is produced, as is the case with so many other Uredineae, is open to question, and the view is gaining ground that the fungus is homoecious.

The black rust (*Puccinia graminis*) forms darker masses of uredospores in longer sori and the teleutospores are produced in characteristic linear pustules surrounded by the torn edges of the epidermis of the host. As is well known an aecidial stage occurs on the barberry. The infection of wheat is readily brought about by uredospores or by aecidiospores, either of which produce crops of uredospores. The details of infection are very similar to those of the yellow rust.

The biologic form of *Erysiphe graminis* on barley forms a web or felt-like coating on the stems and foliage which at the period of spore formation becomes pulverulent. The fungus is distributed throughout the summer by conidia which on germination give rise to an epiphytic mycelium attached to the plant by haustoria driven into the epidermal cells. No deep-seated mycelium similar to that of the rusts is produced.

A considerable collection of both wheats and barleys was obtained and in order to select the most suitable varieties for this investigation the incidence of these three parasites was observed in detail during the year 1902. A similar series of observations had previously been made in Sweden by Eriksson[1], but in view of the fact that it is often stated that susceptibility to disease varies with climatic conditions it was thought necessary to cultivate and test the varieties under the conditions which would obtain during the experiments. In Eriksson's trials the varieties are grouped into five classes ranging from 0 with no disease to 4, very badly diseased. The attempt was made to classify

[1] Eriksson, *ibid.* pp. 333 and 342.

the series in a similar way. In all cases where Eriksson placed a variety in either of the extreme classes the results were the same on my plots, but there was often a difficulty to decide between classes 2 and 3 and 3 and 4. Where no actual standards for comparison were available this was only to be expected and the general impression which remained was that Eriksson's classification agreed well with the one made under the conditions obtaining on the Cambridge plots.

From amongst the series representatives of each class were selected and these have been kept under cultivation since, the remainder, except a few required for other experiments, being discarded. Since 1902 the rust epidemic has varied considerably in its intensity, but the varieties have still retained their relative positions on the arbitrary rust scale with one possible exception. This was a variety known as New Era which originally was placed in class 1, *i.e.* slightly susceptible. In 1906, a year in which there was comparatively little rust, it remained practically rust free until the foliage was beginning to die, when pustules appeared.

The immune varieties chosen were Einkorn, Hungarian Red and "American Club." Each of these has been kept under continuous observation, and in order that there should be no question of their not being exposed to infection they have been grown in company with the susceptible forms whose rust-coated leaves have continually overlapped them. In addition, once or twice each season they have been watered overhead with a fine spray containing quantities of uredospores in suspension.

Einkorn (*Triticum monococcum vulgare*, Kcke). This variety tillers to an enormous extent, forming a thick turf of foliage. It is notoriously rust-resistant though this dense mass of foliage would seem to provide excellent opportunities for infection. In Eriksson's trials it was the only variety immune to the three common wheat rusts. Reports from various parts of the world show that this characteristic is retained under various climatic conditions. On the Cambridge University Farm the variety has been entirely rust free each season with the exception of 1906 when on some fifty plants two leaves were found late in the season on which a few scattered pustules occurred. The majority of these were unbroken and shed no spores before the death of the leaves.

Hungarian Red. The grain of this variety was obtained under the name of "Ungarischer roter" from Haage and Schmidt of Erfurt. It is probably identical with the variety examined by Eriksson. In his

trials it proved immune to *P. glumarum*, but not to *P. dispersa*, except in the rust year 1892 when it was slightly attacked. In the trials at Cambridge it has been perfectly free from the yellow rust but no statements can be made as to its susceptibility to the attacks of other species, for their appearance during the last four seasons has been too spasmodic to ensure fair tests. It has proved a feebly growing variety and its development is often crippled by attacks of *Erysiphe graminis*.

"American Club." This is a variety of *Triticum compactum* which so far I have been unable to identify with certainty. Its morphological characters agree with those of Bearded Herisson or Hedgehog wheat, but whereas this is susceptible to yellow rust the American Club is immune. It was obtained from Mr A. E. Humphries, who found it growing in a plot raised from a commercial sample of Northern Duluth wheat. This, as usual, was graded wheat and among numerous other varieties contained a small proportion of the variety in question. By chance my culture was sown between plots of Michigan Bronze and Hungarian White, another highly susceptible wheat. Throughout the late spring and summer of 1904 its plot was a vivid green colour contrasting markedly with the bright orange yellow of its neighbours which were so badly infected that the soil below them was tinged with the masses of uredospores produced. Late in the season the plants were covered with aphis and the coating of honeydew provided a substratum for the growth of *Cladosporium herbarum*. Yet though the plants were continually exposed to infection and in the latter part of their development under peculiarly unfavourable conditions for healthy growth they remained entirely free from yellow rust.

Since then some three hundred plants have been grown and kept under close observation and only two immature pustules have been found. These occurred in August 1906 on leaves again covered by aphis. The pustules did not break through the epidermis and it is impossible to be certain, though on the whole it is probable, that they were yellow rust. In colour they were slightly darker than normal yellow rust, but their almost circular shape would distinguish them from the more linear pustules of black rust.

It is probable that this variety is susceptible to both black and brown rusts but so far the evidence to hand is not complete[1].

[1] Since writing the above, reports from the Transvaal and from Canada, where small sowings of this variety have been made, show that it is very susceptible to the black rust. It is possible that this is the wheat known as Kidd in Canada, which is similar morphologically and known to be susceptible to this rust.

In describing these varieties as immune it is essential that attention should be called to the fact that they are immune under the ordinary conditions of cultivation of wheat. Absolute immunity under all conditions is not to be expected in any wheat where the rusts are in question. In fact it is practically impossible to say that any plant is immune to the attacks of wheat-rusts if the preliminary stages of infection are to be taken as evidence of susceptibility, for the uredospores germinate on the majority of phanerogamous plants and push hyphae through the stomata into the intercellular spaces[1]. Under such circumstances no further development occurs and the mycelium perishes.

Further emphasis has to be laid on the importance of natural conditions for the development of the host plant, since Salmon[2] has shown that barley foliage may be infected with the biologic form of *Erysiphe graminis* found on wheat if only it is exposed to sufficiently drastic conditions. To secure infection the leaves have to be chloroformed, scorched, bruised, etc. and when their vitality is sufficiently lowered the mildew from the wheat succeeds in getting a hold and developing sufficiently to produce a feeble crop of conidia. Yet under normal conditions infection is impossible. As a matter of fact in the three varieties selected for these experiments the early stages of infection are readily observable in preparations made from foliage growing in the open, but the growth of the mycelium is quickly inhibited and it dies without causing any noteworthy injury to the host. As will be shown later there is also the possibility that infection may occur on foliage which is passing over to a moribund condition. Details of the histology of infection are published by Miss Marryat in this number of the Journal.

Of the slightly susceptible varieties which have been crossed with immune types the more important are Street's Imperial (Class 2) and New Era (Class 1). These grown under the same conditions as the immune varieties have each season produced numbers of sparsely scattered pustules on all of their leaves. In 1906, a poor rust year, the infection was less marked than usual and only occurred late in the season. Of the two, Street's Imperial is the more susceptible and it may be taken to represent roughly the extent to which the majority of our English varieties are susceptible. The varieties characterised by

[1] De Bary, *Comp. Morph. and Biology of the Fungi*, p. 361. Gibson, *New Phytologist*, Vol. III. p. 184.

[2] Salmon, *Anns. Bot.* Vol. XIX. p. 125.

excessive liability to infection which have been used as parents are "American 1," "American 2," Preston, Hungarian White, "Tasmanian" and Michigan Bronze. These are placed as far as possible in the order of their susceptibility.

"American 1 and 2" are varieties grown from graded Northern Duluth and Manitoba wheat. Both are varieties of *Triticum vulgare* but their local names are unknown to me. The following short description may aid in their recognition in Canada and the United States where they are probably commonly cultivated. Number 1 has lax, beardless ears with a yellow chaff: number 2 a similarly shaped ear with white chaff.

Preston is a Canadian wheat of hybrid origin.

Hungarian White, a variety from Haage and Schmidt. This Eriksson puts into classes 4 and 3, stating that it is very susceptible to yellow rust in Sweden. Each of these four varieties throughout the seasons 1903—6 inclusive has been badly attacked and it would have been difficult to count the pustules on any of the diseased leaves. In each case though the plants set a fair quantity of grain.

"Tasmanian" (a variety sent from Tasmania: proper name unknown to me) is very similar to Rough Chaff as far as external appearances go but distinguished from it by extreme liability to the attacks of yellow rust. There is little to choose between this and the succeeding variety in this respect.

Michigan Bronze has earned the reputation of being one of the most susceptible varieties in existence, and on this account it has been largely used by Eriksson in his researches[1]. The pustules generally appear on this wheat earlier than on other varieties and they quickly become so numerous that the whole of the foliage appears to be coated with rust. Every part of the plant is attacked and whereas the upper leaves, chaff and awns of moderately susceptible varieties rarely carry many pustules, in the case of Michigan Bronze these structures are as badly infected as the lower leaves. So severe is the attack that the plants set little or no grain on my plots. In 1906 for instance a plot of forty plants did not produce a single grain. Owing to this difficulty of obtaining seed the stock has to be renewed from year to year and in consequence the plants used for comparison with the hybrids are not, as one would prefer, the direct descendants of the one used as pollen plant. The stock of "Tasmanian" has died out altogether.

[1] Eriksson, *Ann. d. Sc. Nat.* T. XIV. p. 107, 1901.

Some eight hundred plants of these two varieties have been grown since 1902 and not one has escaped infection. So certain is the incidence of the disease that a practice has been made of planting rows of Michigan Bronze among the hybrids in order to ensure the presence of numerous centres of infection. During this last season this precaution was abandoned as unnecessary.

In making the crosses between immune and excessively susceptible varieties the immune parent was in each case the mother plant. Reciprocal crosses were not attempted, since previous experience had shown that there was little likelihood of their succeeding. Where moderately susceptible varieties were under experiment such crosses were successfully attempted.

The following crosses were made between excessively susceptible and immune types:

1. American Club × American 1.
2. „ „ × American 2.
3. „ „ × Preston.
4. „ „ × Tasmanian.
5. „ „ × Michigan Bronze.
6. Hungarian Red × Hungarian White.

The hybridising was carried out in 1904 and the resulting plants were kept under observation throughout the period of their growth in 1904—5. Yellow rust was first observed in the neighbourhood on March 3rd. By May 11th the attack seemed fairly general and wheat in the same field as the hybrids was showing signs of a bad rust season approaching. At this date the hybrids were standing about six inches high. The condition of the plants then was:

1. One plant only: the three lowest leaves badly attacked, the upper leaf still free from rust. This plant was killed shortly after by wireworm.

2. Three plants: a few broken pustules on the lowest leaves, foliage becoming brown and showing numerous dead areas (probably not due to rust).

3. Rust pustules numerous on all the leaves, plant very vigorous.

4. Three plants: all very badly infected with fewer pustules on the highest leaves.

5. Nine plants: the extent of infection differed on individual plants:

(*a*) leaves as far as the fourth one badly infected.
(*b*) leaves as far as the second one badly infected.
(*c*) leaves as far as the fourth one badly infected.
(*d*) leaves as far as the fourth one badly infected.
(*e*) rust on first and second leaves only.
(*f*) on the three lower leaves, upper quite free.
(*g*) infected badly as far as the fourth leaf.
(*h*) almost rust free: only a few scattered pustules on the lower leaves.
(*j*) three lower leaves infected, upper free.

All of these plants were particularly vigorous. Those most badly infected appeared to be diseased to the same extent as Michigan Bronze.

6. Six plants: five of these were uniformly infected up to the fourth leaf, one was entirely rust free. This last plant proved to be a pure Hungarian Red in the next generation, the result of accidental self-fertilization. During June the rust spread rapidly, causing an epidemic of over-average severity. By June 22nd the condition of the plants was as follows:

2. Every leaf dead and covered with the remains of pustules. Death was probably due to other causes besides the rust.
3. Foliage still living and densely coated with pustules.
4. Lower leaves dead, the upper with innumerable pustules.
5. All parts of the plants thickly coated with rust.
6. Very badly infected.

The ears were pushed through the sheaths a week later and the flowering stage was passed. In each of the five series including number 2 the glumes and paleae were becoming infected and pustules had already broken on the awns of the hybrid American Club and Michigan Bronze. At this stage the general appearance of the plants with regard to the amount of rust upon them was very similar to that of the susceptible parents and it was problematical whether enough grain would be secured to carry the experiment on to the next generation. At harvest, however, 4, 5 and 6 yielded a fair crop, far more in fact than the susceptible parents. This may point to the fact that the hybrids were not as badly attacked as the latter and that they were intermediate in this respect, or a possible explanation is that the vigorous growth so characteristic of the F. 1 had enabled the plants to withstand the attack better than the susceptible parents.

Most of the grain harvested was, as was only to be expected, badly

shrivelled but its germinating capacity was fairly satisfactory. It was sown early in the autumn of 1905 under normal conditions of cultivation, part on land which had previously carried a wheat crop, part on soil which was in better condition as it had just previously carried a clover crop. The varying soil conditions made no difference in the final results. The grain from each ear of each plant was sown separately in the case of numbers 5 and 6.

No yellow rust was found on the plots until May 16th, an unusually late date for this district. It occurred on some plots not included in this experiment, but the following day unmistakable signs of rust were found on the series with Michigan Bronze as the parent. By the 29th of this month infection appeared to be fairly general on all the plots. At this date the plants were standing about two feet high and they were in a thoroughly vigorous condition. By June 11th it was judged that any plants susceptible to the disease would have had sufficient opportunity for infection as the plots were fast assuming a bright orange colour and an examination of each individual was commenced.

American Club × Michigan Bronze. Every leaf on the plants, the dead and dying basal leaves included, was carefully inspected and wherever the slightest signs of infection could be detected the plant was entered up in the note-book as susceptible. All diseased plants were cut back to within one foot of the ground whilst the immune plants were left standing to give them further opportunities for infection. The detailed figures for the descendants of plant 5 *a* may be quoted to show the incidence of the disease at this date:

Ear	Rust free	Susceptible
1	9	21
2	9	24
3	9	26
4	8	13
5	17	41
6	7	19
	59	144

Plants 5 *b*, *c*, *d*, and *e* were examined in the same way between the 11th and the 18th of June, the susceptible plants being again cut back. The statistics were as follows:

b	57	141
c	61	128
d	43	147
e	51	157
Total, including 5 *a*	271	717

The plants left standing on these plots were examined at intervals between July 6th and the 16th and a small number of rust susceptible plants were found:

		Revised totals	
	Rusted	Free	Rusted
in 5 *a*	3	56	147
5 *b*	12	45	153
5 *c*	14	47	142
5 *d*	7	36	154
5 *e*	7	44	164
	Total	228	760

In the meanwhile the foliage of the remaining plots of this series was turning yellow and dying off and teleutospores were being formed. As there appeared to be no possibility of susceptible plants having escaped infection at this date the plants were pulled up by the roots in order to make the examination more rapidly, some fifty infected ones being left for seed. Plants 5 *f*, *g*, *h* and *j* gave a total offspring of 1244, 295 of which were free from yellow rust and 949 badly infected. The whole series thus yielded 523 immune and 1609 susceptible individuals.

The immune plants which had been left standing remained green longer than the diseased ones, and their foliage did not commence to turn colour until July 24th. The leaves remained flat and polished and the straw was either a vivid red or yellow colour very unlike that of the neighbouring plots, which, as is the case with most wheats, turned a dull brown owing to the attacks of *Cladosporium herbarum* following the rust. The immune plants could readily have been sorted by the brilliancy of their colour alone.

Whilst the foliage was dying during the last week of July the immune plants were again examined in detail. They were now surrounded by strongly growing branches thrown up by the plants previously cut back and these were carrying an unusually heavy crop of uredospores. The plants were thus exposed to infection at a time when as a rule no uredospores are being formed on wheat. In addition to yellow rust these new branches were infected with both black and brown rust. Symptoms of infection were found on at least twenty of the immune plants; the symptoms being either discoloured areas on the bright yellow ground or in some cases distinct pustules. In all cases these pustules were small and unbroken and they had shed no uredospores when the leaf shrivelled. It is impossible to say whether

these immature pustules were those of *P. glumarum* or one of the other cereal rusts. On the whole I am inclined to think that they belong to the former species, and that when the chlorophyll contents were broken down, foodstuffs translocated for the finishing grain and the vitality of the leaf exhausted it became a prey to the fungus[1]. Assuming these immature pustules were those of the yellow rust this is not an unreasonable explanation though it is a little strange that no pustules could be found on the dying basal leaves earlier in the season. These twenty plants have not been included in the total of rust-susceptible plants.

In addition to the series described, about a hundred plants of the same parentage have been used for infection experiments and for pot cultures for demonstration at the Derby Show of the Royal Agricultural Society and at the Conference of Plant Breeders at the Royal Horticultural Society. No statistics of the number of immune and susceptible plants have been kept in these cases.

Concurrently with this segregation into immune and susceptible forms there was of course the usual segregation of other Mendelian characters. The two parents do not show many pairs of differentiating characteristics, the most important pair being lax and dense ears. There are slight foliage differences but these are not marked enough to discriminate with any certainty. The hybrid plant had dense ears which at first sight were very similar to those of the American Club, but which on measurement were found to be intermediate between lax and dense. The whole habit of the plant, excluding its susceptibility to yellow rust, was very similar to that of the mother parent. In the following generation lax, intermediate and dense eared individuals, occurred in, as a small trial count showed, the usual ratio of 1 : 2 : 1[2]. The immunity and susceptibility were distributed impartially over each of these groups. The plants with lax ears were similar to Michigan Bronze, and those with dense ears to American Club, so that immune types of Michigan Bronze and susceptible types of American Club have been bred. In other words the factors which determine immunity or the reverse are transmitted as Mendelian characters.

Whether this is due to the presence of toxins and antitoxins, as seems probable, further research will have to decide.

[1] It is a well known fact that fungi can readily attack withering or even resting plant tissues which in a normal or an active state are immune. Hartig for example has shown that the mycelium of *Peziza Wilkomii* can only work its way through the tissues of the larch when in a resting condition.

[2] Spillman, see *Journ. Hort. Soc.* Vol. XXVII. p. 876.

From the statistics already quoted it is clear that if all plants which have become infected are grouped as susceptible, and those which have not as immune, then immunity is recessive to susceptibility, for they occur in the ratio of 1 : 3·07, a sufficiently near approximation to the ratio of 1 : 3. If this is the case, then, in the succeeding generation all the immune types will breed true to this character, while the susceptible types will breed true in the proportion of one in three. This part of the subject has still to be tested in this particular series, but in this connexion it may be noted that in a previous experiment the extracted (practically) immunes bred true to this character whilst the dominants split in some cases and bred true in others[1].

During the sorting of the plants into the two groups, however, another possibility presented itself. The extent of infection was obviously very different on different individuals. Whilst some were as badly diseased as Michigan Bronze others were, though by no means free from disease, relatively slightly infected. These latter plants produced considerably more grain than the very susceptible forms.

Thus fifty ears of the lax type taken at random produced 0·8 grms. where the plants were excessively susceptible, 64 grms. when moderately susceptible, and 145 grms. when immune.

These facts seem to point to the existence of intermediate liability in the heterozygote, for without question the less susceptible types were in the majority. The method adopted of cutting out each plant showing infection was not satisfactory for obtaining statistics of this sort, since intermediates would be grouped with extremely susceptible plants. If these indications are correct, and next season's tests will settle the matter, then instead of dealing with ordinary dominance the existence of intermediates will have to be recognised in the probable proportion of one immune and two intermediates to each extremely susceptible type.

Hungarian Red × Hungarian White (6)[2]. The F. 2 again consisted of extremely susceptible and immune individuals. A detailed examination of the series was not made until the third week in July, when the foliage was beginning to show symptoms of dying off. Twenty-four plants were entirely free from yellow rust and the remaining 109 were attacked to about the same extent as the susceptible parent.

American Club × American 1 (1). The grain from the F. 1 plants

[1] Biffen, *Journ. Agric. Sci.* Vol. I. p. 43.

[2] The statistics for this series were obtained by Mr S. V. Shevade of the Pusa Research Station, India.

did not in this case germinate satisfactorily, a sowing of about 250 only giving 83 plants. The majority of these were excessively rusted and many succumbed to the attacks of the parasite. Counted in the third week of July, 15 were completely rust free, 4 bore incipient rust flecks, and 64 were as badly attacked as the parent American 1.

American Club × Tasmanian (4)[1]. In the F. 2 series there was from the first a considerable difference between the extent of infection on the different susceptible plants and the attempt was made to grade them into immune, moderately susceptible and excessively susceptible forms. The first examination was made on June 19th. The number of diseased and disease free plants was then approximately equal, there being 39 of the former, and 37 of the latter. Of the 39 rusty individuals, 21 were moderately and 18 extremely susceptible. In the former group the pustules only occurred on the lower leaves whilst in the latter all the leaves were attacked. A second examination made on July 10th, showed no readily recognisable difference between the moderately and extremely susceptible individuals, though it is possible that if the count had been made before the heavy rains of the preceding week, which had washed the pustules clean, the two classes could have been distinguished. The final count gave a total of 56 rusty and 18 completely immune plants (two plants missed).

In the above cases one of the parents has been chosen for its excessive susceptibility to the attacks of yellow rust, whilst in those to follow slightly and moderately susceptible parents have been used in conjunction with an immune one. These less susceptible varieties were New Era and Street's Imperial. The former would, I think, be placed in Eriksson's class 1, the latter in class 2. In 1906, New Era was almost entirely free from rust until the end of the season, whilst Street's Imperial was only slightly attacked. Both varieties were crossed with American Club, and the F. 1's raised in 1905 matched the susceptible parent in the extent to which they became attacked.

The first generation from the hybrids of American Club and New Era remained almost rust free, and no attempts were made to obtain any statistics as to the incidence of the disease on the plots until the middle of July. A trial count then showed that the infected plants were roughly equal in number to the immune ones, and the further examination was delayed until the end of the month when the foliage

[1] S. V. S.

was showing symptoms of dying. The infected plants at this date showed few pustules on their foliage and none on the glumes and awns. Many had unbroken pustules and pale yellow flecks which possibly indicated abortive attempts at infection. All plants showing pustules were counted as diseased, with the result that 135 were placed in this class and 100 in the completely immune class. The plot contained about six hundred plants, but it was not thought worth while to examine the whole series. A comparison with the New Era at this period showed that the infection was unusually slight, and several of the plants only showed yellow flecks and no pustules. For some reason, probably connected with climatic conditions, the intensity of the attack was far less than it had been in previous years. This tendency to remain free from infection appears to have been retained in the F. 2 generation.

In the case of the hybrids with Street's Imperial and American Club, infection occurred early in the season, and there was from the first an obvious difference between the immune and susceptible individuals. When examined in detail on July 18th, 62 diseased plants and 22 immune plants were found on the plot.

One cross between two varieties, both susceptible to infection but differing in the intensity of the attack, has been kept under observation. The parents in this case were Rivet wheat and Emmer or *Triticum dicoccum*. The Rivet parent has for the last five years been slightly susceptible, and the rust has always been late in appearing on it. The Emmer is classed by Eriksson in group 2 in two seasons and group 3 in a third. No details with regard to its rustiness have been kept as the cross was originally intended for a study of the grain characters only. From the general agreement between my observations and Eriksson's with regard to the extent of the disease, it may be assumed that the rustiness of this variety would be the same in Cambridge as in Sweden. The F. 1 grown in 1905 was moderately susceptible to disease, and part of the F. 2 generation on June 20th consisted of 58 immune plants and 68 susceptible. On July 7th the whole plot was examined with the result that 204 plants were classed as rusty, 48 with traces of rust, and 23 immune. Tags were affixed to the immune plants in order to harvest them separately, as it was considered at this date that as they had resisted infection so long, they were probably really immune. The plants retained the green of their foliage longer than most of the hybrids (a characteristic of both parents) and during the first week in August it was found that those previously

marked as immune had become infected and bore pustules which had broken the epidermis. The totals thus became 204 moderately, and 71 slightly infected. The Rivet wheat was then examined and pustules were found on each of the 20 plants grown as a control, though the plants were free at the time at which the second examination of the hybrid plot had been made. Here as in the case of New Era and American Club the period at which infection occurs appears to be inherited as well as the susceptibility itself.

In addition to the series of F. 2 experiments already described, further generations of other crosses showing differences in the susceptibility of their parents have been kept under observation. In one case only was the cross made for this particular purpose, namely that between Red King and Rivet wheat already referred to. The extracted recessives only have been grown, and in the F. 4 they have retained their characteristic rust-resisting capacity unimpaired. The plots could be matched against the parent Rivet satisfactorily in this respect.

In a second case an extensive trial of a number of fixed types resulting from crosses between a susceptible Manitoban wheat and the far less susceptible varieties Rough Chaff and Lammas was being made for other purposes. Each of the plots of some forty of these fixed types contained some thousands of individual plants. Early in June symptoms of the coming rust epidemic were observable, and by the end of the month many of the plots were badly attacked, whilst their neighbours of the same descent, growing under identical conditions, were relatively free from disease. Two typical plots were examined in detail: in the first case every plant was found to be badly attacked, and the extent of the disease corresponded accurately with that of the Manitoban parent growing in a plot near, whilst in the other all the plants were slightly infected only. The extent of the disease probably corresponded with that of the relatively immune parent, though this could not be satisfactorily observed as no plot of this was grown under the same conditions. These plots represented all the possible combinations of the morphological characteristics of the parent varieties, and they afforded a striking field demonstration of the fact that there is no correlation between such characters and liability to the attacks of the yellow rust.

This was further illustrated in the case of a cross between Polish and Rivet wheat. The former has proved slightly more susceptible to the attacks of yellow rust than the latter, though the difference is by

no means as pronounced as in the case last described. A number of fixed types in the F. 3 generation were being tested for other purposes in 1905, and examples were repeatedly met with in which types as far as could be determined, absolutely identical with one another morphologically, differed markedly in rust resistance. Trial sowings in the following season showed that this peculiarity was retained.

Without doubt numerous other similar cases could have been found if all the hybrids then being cultivated could have been examined for these characters. Unfortunately in many cases no notes had been made as to the susceptibility of the parents, and the F. 1 and F. 2 generations had not been kept under special observation.

The attempt to determine whether the inheritance of immunity to the attacks of *Erysiphe graminis* was similar to that of yellow rust was made with both wheat and barley. The case of barley only will be considered here as the F. 2 generation of the wheat hybrid failed to become infected. The parent barleys were *Hordeum spontaneum* (Koch) and *Hordeum hexasticofurcatum* (K. H.). The former is as a rule completely free from mildew, whilst the latter is the most susceptible variety which could be found in a collection containing some 140 varieties. The crosses were, as in the case of most of the wheats, made on the immune parent. The resulting hybrid was attacked by the mildew, to, as far as one can judge such matters, the same extent as the susceptible parent. Its grains were sown in the spring of 1905 and gave a plot containing 79 plants. The Erysiphe did not appear until late in the season, though it was fairly abundant on field plots in the neighbourhood at an early date. The late infection may possibly have been due to the fact that the plants were unusually vigorous owing to their having been planted in garden soil at wide intervals. Fearing failure through lack of opportunity for continuous exposure to infection, artificial inoculation was resorted to. Leaves covered with mildew from a number of varieties were shaken up in water and the whole plot sprayed with it and then shaded for two days. This had the desired result, and a week later the whole plot was thick with mildew. On July 12th a detailed examination of the plot was made. The majority of the plants were very badly attacked, some bore traces of mildew, and a few were altogether immune. Those with traces of mildew and the immune individuals were kept under observation until the plants were beginning to show signs of the foliage drying preliminary to ripening, and at this stage the three types were sorted

out and counted. Fifty-six were badly attacked, 16 bore traces of mildew, and seven were altogether free from it.

At this time the parent *Hordeum spontaneum* was very slightly attacked, though no signs of disease had been seen in the two previous seasons, the severity of the attack corresponding with the traces of mildew found on some of the hybrids. The second parent *H. hexastico-furcatum* was badly diseased and matched the majority of the hybrids in this respect. No further generation of this cross has yet been grown.

The experiments with *Puccinia graminis* have not been altogether successful. The first attempts were made with Rivet wheat and Einkorn, the latter being used as the male parent in order to simplify the operation of crossing. Three strong F. 1 plants were raised in 1904, and as no black rust had appeared on these at the end of June, the plants were inoculated with aecidiospores from the barberry. Ten days after each plant was showing pustules which increased enormously in numbers, and by the time of harvest the straw was blackened with the gaping teleutospore sori. A few shrivelled grains were produced but these all failed to germinate, and in consequence no F. 2 generation was raised. The extreme susceptibility of the hybrid is rather unexpected, for Rivet wheat has not up to the present proved itself unusually so. A second F. 1 generation of this same cross has been raised, and this has entirely escaped infection with black rust, though slightly attacked with the yellow rust.

From analogy with the cases already described and from the susceptibility of the F. 1, it would appear that the susceptibility to black rust is also a dominant character.

From the foregoing series of experiments it is evident that the inheritance of immunity and susceptibility to the attacks of certain parasitic fungi can be traced as readily as that of morphological characters, and that immunity is recessive to susceptibility. Whether this is generally true or applies only to these special cases still has to be determined, but, for the time being, the fact that disease-resistance is recessive may be employed in attempts to cope with the losses due to the attacks of rust in cereals. Wherever these crops are cultivated the rusts take their toll, causing in the aggregate enormous losses. Thus in the rust year, 1891, Prussia alone is stated to have lost some £20,600,000, or approximately two-thirds of the value of the entire cereal crop[1], and according to M'Alpine[2], "at a

[1] *Zeits. für Pflanzenkrankheiten*, 1893, p. 185.

[2] M'Alpine, *Victorian Naturalist*, Vol. XXIII. p. 45.

low estimate it is considered that £100,000,000 does not cover the annual loss (due to rusts) to cultivated cereals alone."

The outcome of the numerous attempts made to minimize these losses is that prophylactic measures are useless, and the one hope left is to grow in each country varieties which prove rust-resistant. It has been known from comparatively early times that some varieties are more resistant than others. Thus Jethro Tull points out that "white cone or bearded wheat...is less subject to blight than Lammas wheat, which ripens a week later." In some countries a careful search has already been made for rust-resistant varieties, but on the whole, with comparatively little success from the economic point of view. This partial failure has not been due so much to the difficulty of finding relatively immune varieties, as to the difficulty of finding immunity in combination with other features essential for the profitable cultivation of the crop. Knight[1] appears to have been one of the first to realise the necessity of the "formation or selection" of resistant varieties, and from time to time the attempt has been made. The researches of the late William Farrer may be quoted as an example[2]. The problem has proved an exceptionally difficult one, and even Farrer's patient work has not met with the success one hoped it would. Now, however, that we are in the possession of the broad outlines of the inheritance of the more important characteristics of wheat, the attempt to combine in one variety such features as quality, proper time of ripening, cropping power and so on, together with immunity to the commoner rusts, may profitably be made. Such attempts will have to be made in each country where wheat is cultivated; for wheats suitable for English conditions will certainly find no favour in Canada or Australia for instance. Under such circumstances it would be useless for a breeder, knowing one set of conditions only, and that one not particularly suitable for experiments of this kind, to attempt to do more than indicate the mode of attack which appears to be most suitable. In the first place, the varieties most suitable for the locality will have to be selected for crossing with any which are found to be more or less rust-resistant. From Eriksson's researches it is clear that some varieties are resistant to one rust but not to others, and where this particular rust happens to be the serious one locally, such a variety may be used for breeding experiments. There is, however, one wheat which is characterised by exceptionally complete immunity to the attacks of the

[1] Knight, *The Pamphleteer*, Vol. VI. p. 402, 1815.
[2] Farrer, *Agric. Gaz. of New South Wales*, Vol. IX. p. 131, 1889.

three common rusts—the yellow, black, and brown, namely, *Triticum monococcum* or Einkorn. This primitive type will in all probability prove in the long run the most valuable source of immunity. There is, however, one drawback to its employment for this purpose, that being its complete lack of all the characteristics which go to the building up of a typical bread wheat. Consequently the breeder has to face a somewhat complex problem, but knowing the characters of each of the varieties used as parents, he can calculate the chances of obtaining the combinations required, and by growing sufficiently large cultures of the generation bred from the hybrid, make certain of their occurrence. From the experiments already described it may be inferred that the immunity is transmitted in all its entirety so that any bread wheat with Einkorn as one parent might then be handed on to the other workers to simplify the problem of raising varieties suitable for other districts.

Summary.

On crossing immune and susceptible varieties the resulting offspring is susceptible.

On self-fertilization these susceptible individuals produce immune and susceptible descendants in the proportion of one of the former to three of the latter. The degree of susceptibility is variable.

Where the degree of susceptibility differs in the two parents the hybrid resembles the more susceptible parent in that respect. Among the descendants of such hybrids the two degrees of susceptibility appear in the usual Mendelian ratio of one slightly to three very susceptible individuals.

The relatively immune forms breed true to this characteristic in the succeeding generations.

Immunity is independent of any discernible morphological character, and it is practicable to breed varieties morphologically similar to one another, but immune or susceptible to the attacks of certain parasitic fungi.

NOTES ON THE INFECTION AND HISTOLOGY OF TWO WHEATS IMMUNE TO THE ATTACKS OF *PUCCINIA GLUMARUM*, YELLOW RUST. [WITH PLATE II.]

BY DOROTHEA C. E. MARRYAT.

IT has been known for a long time that there exist varieties of wheats and other cereals which appear to be practically free from, or immune to, the attacks of certain fungal parasitic pests, by which closely allied forms are ravaged.

As far back as 1815, Thomas Andrew Knight suggested that these forms should be carefully sought out and cultivated by farmers[1]. Mr Biffen, on the University Farm at Impington, Cambridge, has for some years past given special attention to this subject. He has discovered and grown several wheats which show to a greater or less degree immunity to the attacks of *Puccinia glumarum*, Yellow Rust. He has further proved that this power of resisting disease behaves as a simple Mendelian character, so that on crossing such wheats with those which, though non-immune, possess other desirable qualities, the latter can be combined with freedom from disease, and a pure race eventually bred[2].

In applying the term "immune" to a plant, it must be explained that this does not signify that it is never attacked at all by the parasite, but rather that the latter is unable to develope normally in such a host, and that since its progress is checked, and spores are never, or only rarely produced, the plant is practically unharmed and infection cannot spread.

The late Professor Marshall Ward and Mr Evans a few years ago examined the histology of one of these wheats, but published only a very brief account of the results which they obtained, without giving any figures[3].

[1] *Pamphleteer*, Vol. VI. p. 402.

[2] Biffen, *Journal of Agricultural Science*, Vol. I. p. 40.

[3] Marshall Ward, *Annals of Botany*, Vol. XIX. p. 35.

Since then, several wheats have been discovered by Mr Biffen which display a considerably greater degree of immunity than the one then used (Rivet Wheat). I have recently examined several of these and here give a rather fuller account of the phenomena observed.

Two forms were used for experiment: (i) *American Club* (a variety of *Triticum compactum*) obtained by Mr Biffen from Mr A. E. Humphries, who found it growing in a plot raised from a commercial sample of N. Duluth wheat, together with several other varieties. (ii) *Einkorn* (*Triticum monococcum vulgare*, Kcke), a half-wild form, which except for this character of immunity, is practically worthless from a farmer's point of view. Of these two wheats, *Einkorn* shows in the field an even greater degree of immunity to Yellow Rust than *American Club*, for only in two instances have a few small scattered pustules, which had not broken the epidermis, been found upon its leaves, in spite of the examination of hundreds of plants[1]. For purposes of comparison, cultures were also made of a third form, *Michigan Bronze*, a wheat peculiarly susceptible to the attacks of Yellow Rust.

Details of Infection.

In June 1906 a number of seedlings of these three wheats were raised in flower-pots, in a cool greenhouse. They were covered with bell-jars in order to prevent accidental infection from stray spores. When the young plants were well up, a number of leaves (which were then marked) of each of the three forms, were infected with uredospores from the fresh leaves of adult plants of *Michigan Bronze*, which were literally orange with Rust.

No change was noticeable in the seedlings until the 6th day after infection, when two leaves of *Michigan Bronze*, the highly susceptible form, showed the first signs of disease, namely, pale yellow flecks. On the following day, almost all the *Michigan Bronze* leaves which had been infected showed similar discoloured areas; they were also found on two of the *Einkorn* and three of the *American Club* seedlings.

After this day, the infection continued to spread rapidly amongst the *Michigan Bronze* plants but more slowly amongst those of *Einkorn* and *American Club*.

By the 11th day, the state of affairs was as follows: almost all the *Michigan Bronze* leaves which had been infected—some 39—showed long, yellowish disease areas, whilst on four of these were visible

[1] Biffen, *Journ. Agric. Sci.* Vol. II. p. 112.

numerous small, but distinct, orange pustules. In *Einkorn*, on the contrary, disease areas were found in only 12 out of the 37 leaves originally infected, and these areas were deep yellow or brownish, and quite small. On some of them were noticeable little black flecks, looking as though the leaf-tissue had been scorched with a red-hot needle. There was no trace of pustules.

In the *American Club* leaves, the infection had spread more than in those of *Einkorn*, but here also, the diseased areas were of a deeper yellow, and indeed the leaves looked more unhealthy than in *Michigan Bronze*, although the actual disease areas were larger in the latter. (The reason of this will be understood when the histology is described.) Further, there were no pustules.

Two days later (13 days after infection), all the infected leaves of *Michigan Bronze* except 5 showed numerous well-developed pustules, in spite of which the leaves looked fairly healthy.

In *Einkorn*, the infection had hardly spread at all, only 3 more leaves—making 15 in all out of the 37 originally infected—showing quite small, deep yellow areas. There was still no sign of pustules.

In *American Club*, the disease areas had spread somewhat, and were visible in most of the leaves which had been infected, whilst on one leaf, when examined with a lens, were found a few scattered, feeble-looking pustules, very different in appearance from those seen in *Michigan Bronze.*

By the 16th day, every leaf of *Michigan Bronze* which had been infected, save one, was orange with pustules throughout its length, and stood out in the most striking manner amongst the remaining green leaves upon which spores had not been sown.

In the *Einkorn* plants, little change was noticeable; no more leaves showed signs of infection, and in those in which the fungus had managed to make good its entry, it had apparently received a severe check by the withering and death of the leaf-tissue immediately in its vicinity, and was unable to spread farther. The remaining portions of the leaves attacked, appeared for the most part green and healthy. On the 18th day however, one rather shrivelled leaf showed a few scattered, unhealthy-looking pustules, none of which appeared to have burst the epidermis. The pustules did not spread, and were not observed on any other *Einkorn* leaf.

American Club offered an intermediate case between *Einkorn* and *Michigan Bronze*, for although the infection had spread to a certain extent, and by the 17th day, 10 leaves on about 50 plants showed a few

of the minute scattered pustules already referred to, they were so small and insignificant as to be scarcely noticed unless hunted for, and bore no comparison whatever, either in number or size, to those produced on *Michigan Bronze.* During the next few days they hardly spread at all and had apparently reached the maximum of their development.

The seedlings were finally bedded out in the garden at the Botanical Laboratory and left, to see what their fate would be in the open air. The *Einkorn* and *American Club* plants were arranged in rows between those of the rusted *Michigan Bronze* in order to expose them to every chance of further infection.

When examined in August—about 2 months later—it was found that the leaves of the *American Club* plants showed quite a number of pustules, but that these were not bright orange as in *P. glumarum*, but of a burnt-sienna colour, and were probably due to Black Rust, *P. graminis*, a fungus to which this wheat is not immune, but which since it appears much later in the season than *P. glumarum* does not so seriously affect the crops[1].

Michigan Bronze showed a quantity of the familiar orange pustules of *P. glumarum* on most of its leaves, but mixed with these were also the darker pustules of *P. graminis.* The *Einkorn* plants appeared to have developed no pustules of any kind.

It may at this point be emphasized that these results were obtained with "immune" wheats, when the fungus was given a much better chance of successful development than it would normally have in the field. For in the first place there was a liberal sowing of spores, secondly the infected plants were not only seedlings, but were further weak and much drawn from being grown under glass and consequently less able to resist the attacks of the parasite, thirdly the warm damp atmosphere in which the plants were reared was highly favourable for the germination of the spores and their further development.

Histological details.

Infected areas of all three wheats were cut off at different stages and fixed in Chrom. Acetic solution. Sections were cut with a microtome and stained with Diamant Fuchsin and Light Green. Before describing the abnormal condition of the fungus found in the immune wheats, I will give a short account of its normal development as observed in the susceptible form *Michigan Bronze.*

[1] Biffen, *Journ. Agric. Sci.* Vol. II. p. 118, note.

The uredospores when lying on the surface of the leaves, send out from their germ-pores, germ-tubes, which penetrate into the host through the stoma, and swell out in the spaces beneath the latter into what are known as *sub-stomatal vesicles.* Into these pass the nuclei of the spore. (Fig. 1.) From the sub-stomatal vesicles proceed *infection-tubes* or short hyphae, into which the nuclei pass, and which quickly grow out towards the host cells. (Fig. 2.) The next stage is the production of *haustoria* or suckers, which the young hyphae drive into the host cells, and by means of which they obtain their nutriment. The original nuclei of the hyphae meantime divide rapidly[1].

Successful entry being thus effected, the hyphae increase in size, branch, and spread quickly in the tissues of the host, applying themselves closely to the cells and piercing them by means of numerous haustoria. (Fig. 3.)

In these early stages of the normal life-history of the fungus, three things may be specially noted for comparison with the abnormal development in the immune wheats to be described later.

(i) The *numerous nuclei* present in the hyphae, which take up the red of the double stain and shine out very clearly, the hyphae themselves staining green.

(ii) The *large number of haustoria* sent out by the hyphae into the host cells in their vicinity, two or more being often projected into the same cell.

(iii) The *healthy condition of the host cells*, which are for the most part unshrunken, and well-supplied with chlorophyll-granules, bearing out Professor Marshall Ward's remark that "a Uredine when flourishing in a leaf does not act as a devastating parasite, but as one which slowly taxes its host and even stimulates the cells for some time to greater activity[2]."

The fungus continues to spread rapidly throughout the leaf until about the 10th or 11th day, when small hyphae begin to mass themselves beneath the epidermis, preparatory to the formation of pustules. The nuclei now arrange themselves in pairs along the length of the hyphae, which become divided up by septa. Before long, the proximal end of each hypha begins to swell, assumes a more or less oval shape and is finally abstricted as a uredospore. That end of the hypha from

[1] The hyphae of *P. glumarum* are unusually large, varying from 3 or 4 to as much as 18 μ in diameter. They are also characterised by showing but few septa and possessing very numerous nuclei.

[2] Marshall Ward, *Annals of Botany*, Vol. XVI. p. 299.

which the spore has been cut off then begins to swell up to form another, and so on until a whole mass are produced, which soon rupture the epidermis and escape, to repeat the life-cycle when they fall upon a suitable host leaf. (Figs. 4 and 5.)

The young spores, before they are abstricted, stain dark green, and usually show the two red nuclei very beautifully (Fig. 4), but as they mature, they become filled with the oleaginous granules which give the pustules their bright orange colour; the spores then stain dark red, and the nuclei can generally no longer be made out. Germ-pores, through which the germ-tubes will later be put out, are visible in the ripe spores. (Fig. 5.)

It has already been stated that by the 12th or 13th day the *Michigan Bronze* leaves were covered with pustules. These present in section a very characteristic appearance. The tangles of green hyphae from which have been cut off countless red spores, stud and finally burst both the upper and lower epidermis. (Fig. 6.)

After this sketch of the normal development of the fungus, the condition of the hyphae in the immune wheats may be considered. *Einkorn*, as offering the more extreme case, will be described first.

Entry takes place as usual through the stoma, but almost from the beginning the contents of the hyphae look watery and show very few nuclei or merely finely-granular red-staining areas, which perhaps consist in part of broken-down nuclei. (Fig. 7.) Still more striking than this unhealthy appearance of the hyphae is the fact that they appear too feeble to send out any haustoria, for only in a single instance were two very minute ones observed. (Fig. 8.) Already, even in the early stages, the host cells in the vicinity of the fungus look shrunken and contain few chlorophyll granules, whilst in some places, they are already beginning to break down. Rather later stages show parts of the leaf-tissue reduced to a dead shrivelled mass, in the midst of which lie numerous large hyphae, apparently also dead, and stained a uniform deep red. (Figs. 9 and 10.)

Such completely dead areas correspond no doubt to the scorched-looking flecks already noticed on the growing leaves. And it is the more striking to observe them in section after section when one reflects that in a normal case of infection, the leaves would by this time be a mass of pustules.

A word may here be said as to the characteristic manner in which these dead hyphae take up the red stain. It is undoubtedly due to the lack of sufficient nutriment and the consequent formation of oil drops

and other fatty products of starvation. For it has been shown that if the hyphae in an ordinary "non-immune" wheat are starved by cutting off the carbohydrate supplies of the host, they also become highly-granular and stain deep red in the manner described above[1].

It has been mentioned that on one single leaf of *Einkorn* a few pustules were formed. Sections of this leaf proved very interesting. Several normal, though rather small pustules were found, but not even the healthiest-looking had managed to burst the epidermis. Mingled with them were numerous "attempts" to form pustules, if one may so speak, attempts which had obviously proved unsuccessful. The weft of hyphae composing such abortive pustules, was in most cases very small and confused, and instead of being close beneath the epidermis, lay deep down in the leaf-tissue. Moreover the spores, if spores they can be called, were very small and ill-shaped and scarcely recognisable as such. (Fig. 11.)

To sum up, it seems that in this immune wheat, although the fungus manages to make good its entry, to produce comparatively large and numerous hyphae, and even in rare cases to arrive at the production of spores, it is sooner or later starved to death by the breaking-down and death of the host tissue in its vicinity and is able to make no farther progress. The host itself, on the other hand, having checked the ravages of its enemy, continues to flourish, except for these small dead areas.

American Club, as already remarked, offers an intermediate case between *Michigan Bronze* and *Einkorn.*

Here again entry takes place normally through the stomata, with the formation of sub-stomatal vesicles etc. Many of the hyphae at first appear perfectly healthy, in that they stain green and show a number of nuclei. Some of them also succeed in putting out quite healthy, normal haustoria, though these are very much fewer in number than in *Michigan Bronze*, and may sometimes be very small.

Before long, however, this successful progress of the parasite is checked. The nuclei of the hyphae appear to become smaller and to degenerate, and the whole contents become highly granular and stain deep red. Such hyphae present a very striking appearance as they lie amongst the green-stained host-cells, and are followed up in section after section. Sometimes one half of a hypha may still stain green and show nuclei, whilst the other half has already begun to undergo this process of degeneration and stains red. (Fig. 12.)

[1] Marshall Ward, *Annals of Botany*, Vol. XIX. p. 39.

Or, again, even hyphae which have managed to put out haustoria may become filled with small granules and their outlines appear so faint and indistinct that they seem on the road to complete disintegration. (Fig. 13.) The host-cells in such cases often appear moderately healthy. But in other portions of the leaf one may find hyphae which are still flourishing, whilst the host-cells in their vicinity are gradually dying in response to a too vigorous onslaught on the part of the parasite. (Fig. 14.)

In other cases, long, highly-granular hyphae stained a deep red are to be seen lying amongst empty, shrunken host-cells (Fig. 15); rarely, hyphae stained a uniform red are found amongst completely broken-up, shrivelled tissue, a condition already described as characteristic of *Einkorn*.

In a word, in *American Club* one is much more conscious of a continued struggle going on between host and parasite than in *Einkorn*, where the rapid breakdown and death of the host tissue in the parts attacked involves the death of the parasite, and thus soon puts an end to the contest.

The invading fungus is by no means flourishing in *American Club*, but though baulked at one point it manages to hold its own at another, until it finally succeeds in forming a moderate number of small, scattered pustules.

Examined in section, however, only a relatively small number of these are found to produce normal spores which succeed in bursting the epidermis. The remainder exhibit for the most part all stages of abortion (Fig. 16), or, if good spores are produced, they do not succeed in bursting the epidermis, and being thus unable to escape, are practically harmless.

Conclusions.

The mutual condition of host and parasite in these immune wheats —as seen on cutting sections—has now been described. And it has been shown that, though the fungus succeeds in making good its entry and producing hyphae, further progress is either completely checked by the breaking-down and death of the host tissue locally accompanied by the starvation and death of the parasite, as in *Einkorn*, or else a more protracted struggle takes place, as in *American Club*. In the latter case the development proceeds to a farther point but is still greatly retarded as compared with a normal case such as *Michigan Bronze.*

But the *reason* of this immunity is still to seek.

It was thought at one time that it might lie in certain structural peculiarities of such wheats, *i.e.* the thickness of the cell-walls, the size and number of the stomata, the nature of the hairs on the epidermis, etc. Professor Marshall Ward has proved by a long series of experiments that no connexion whatever is found, if the curves of such factors are plotted out and compared with what he has termed "infection curves[1]."

We are therefore forced to fall back upon the theory that immunity to disease is due in these cases to the production of certain toxins and anti-toxins by host or parasite or both, which are mutually destructive.

When the problem as to the nature of toxins and anti-toxins has been more completely solved, and more perfect means of isolating them in plants have been devised, some knowledge as to the reason of immunity may be forthcoming. At present one has to be content with a description of the outward and visible effects of immunity, without being able to explain the more subtle cause.

In conclusion, I may say that it was at the late Professor Marshall Ward's suggestion that this investigation was undertaken, and owing to his kindness that I had the privilege of working in his laboratory. I should like to take this opportunity to thank Mr Biffen for kindly supplying me with material and also helping me with advice.

CAMBRIDGE BOTANICAL LABORATORY.
November, 1906.

EXPLANATION OF FIGURES IN PLATE II.

All the figures except 7 and 10 were drawn with the camera lucida under a ⅙ objective (×350) and a 2 eye-piece (×8) [Beck], from longitudinal sections of infected leaves, cut with a microtome and stained with Diamant Fuchsin and Light Green.

Figures 7 and 10 are slightly diagrammatic sketches, based upon camera lucida drawings under a ⅔ objective (×40) and a 2 eye-piece (×8) [Beck].

Fig. 1. Germ-tube, from which the spore has broken off, piercing a stoma, and swelling out beneath into a sub-stomatal vesicle. The latter shows 2 nuclei. (*Michigan Bronze*, 8th day culture.)

Fig. 2. Sub-stomatal vesicle putting out infection-tube or first hypha. The latter shows 4 nuclei. (*American Club*, 13th day culture, a late entry.)

Fig. 3. Older, branched hypha, showing numerous nuclei and penetrating the host-cells by means of haustoria. (*Michigan Bronze*, 7th day culture.)

[1] Marshall Ward, *Annals of Botany*, Vol. XVI. p. 302.

Fig. 4. Young pustule, showing uredospores, each with 2 nuclei. (*Michigan Bronze*, 11th day culture.)

Fig. 5. Mature pustule showing uredospores rupturing the epidermis and escaping. The spores at this stage stain so deeply that the nuclei can no longer be seen, but the germ-pores are visible. (*Michigan Bronze*, 16th day culture.)

Fig. 6. Leaf-tissue studded with pustules. (*Michigan Bronze*, 13th day culture.)

Fig. 7. Long branched hypha which shows no nuclei or haustoria, only slightly granular areas, which stain red. (*Einkorn*, 8th day culture.)

Fig. 8. Short, granular, hyphal branches which have sent out 2 very small haustoria. (*Einkorn*, 8th day culture.)

Fig. 9. On the left is seen the shrunken, dead, leaf-tissue penetrated by hyphae, the whole taking up the red stain, on the right the cells unattacked by the parasite are still normal and stain green. (*Einkorn*, 12th day culture, cp. with Fig. 7, which shows the usual condition found about the same time in a non-immune wheat.)

Fig. 10. Part of a similar "dead" area, showing the large hyphae which stain a uniform deep red. (*Einkorn*, 11th day culture.)

Fig. 11. An abortive pustule. (*Einkorn*, 18th day culture.)

Fig. 12. Branched hypha, one-half still staining green and showing a few nuclei, whilst the contents of the other half have become highly granular and stain deep red. (*American Club*, 7th day culture.)

Fig. 13. Granular hyphae and haustoria undergoing a process of disintegration, outlines of hyphae very indistinct. (*American Club*, 11th day culture.)

Fig. 14. Dead host cell, the walls and chlorophyll granules of which have stained bright red, in contact with a still healthy hypha which has stained green and shows numerous nuclei. (*American Club*, 7th day culture.)

Fig. 15. Long, very granular hypha which has stained deep red, amongst empty, shrunken host cells. (*American Club*, 12th day culture.)

Fig. 16. An abortive pustule of *American Club* (14th day culture).

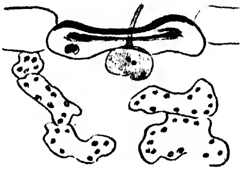


Fig. 1.

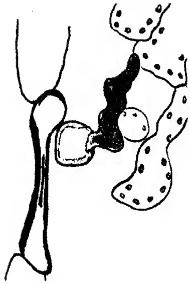


Fig. 2.

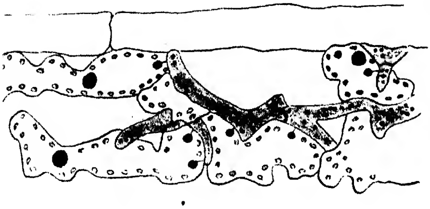


Fig. 3.

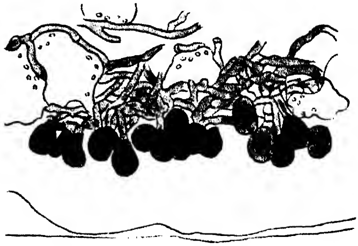


Fig. 4.

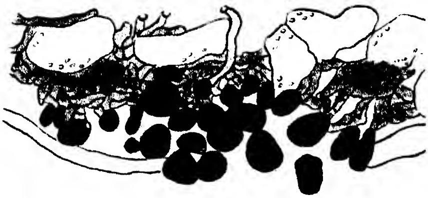


Fig. 5.

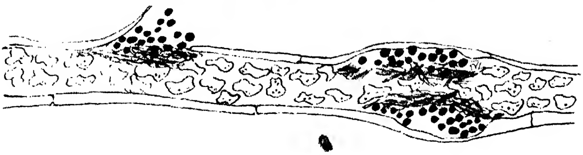


Fig. 6.

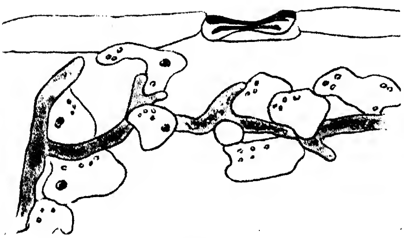


Fig. 7.

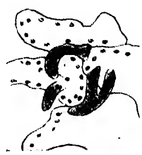


Fig. 8.

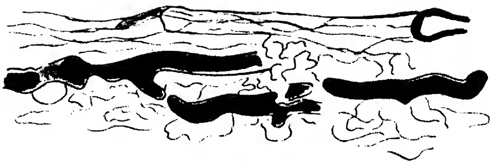


Fig. 10.

Fig. 9.

Fig. 11.

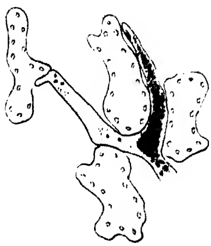


Fig. 12.

Fig. 13.

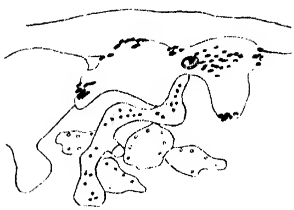


Fig. 14.

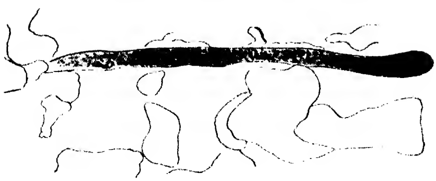


Fig. 15.

Fig. 16.

THE CHEMISTRY OF STRENGTH OF WHEAT FLOUR.

By T. B. WOOD, M.A.,

Reader in Agricultural Chemistry in the University of Cambridge.

Part I. The Size of the Loaf.

THE recent publications of the Home-grown Wheat Committee, and more especially the *résumé* of their work by Messrs A. E. Humphries and R. H. Biffen, which appeared in the last number of this *Journal*[1], have drawn attention to the importance of the wide variation in the baking value of different flours.

The baker likes a flour which will yield a loaf of large size, desirable shape, and good colour and texture, and which can be handled satisfactorily in the state of dough. Another valuable quality to the baker is the power of taking up a large proportion of water in the process of doughing.

It has been the custom, among those concerned with the use of flour, to describe flours which are desirable from the baker's point of view as strong, and since the baker desires so many distinct qualities some confusion has arisen in the use of the term. This was discussed by Humphries and Biffen in the paper referred to above, and the definition of strength they adopt is "the capacity for making large well-piled loaves." Analysing the meaning of this definition it appears that they include in the term strength the separate qualities of size and shape, designedly leaving out the power of absorbing water in doughing, which they quite correctly consider to be an entirely distinct property. Jago[2], on the contrary, defines strength as the capacity of absorbing water, and treats separately the qualities of size, shape,

[1] Humphries and Biffen, *Journ. Agric. Sci.* Vol. II. Pt. I. p. 1.

[2] Jago, *The Science and Art of Bread Making*, London, Simpkin, Marshall & Co., 1895.

colour, and texture. Other investigators are equally at variance in their definitions of strength, and this is perhaps partly the cause of the indefinite state of our knowledge of the chemistry of the subject. In this paper the definition adopted is that of Humphries and Biffen, in which the primary factor is size of loaf, the other factors being shape and, perhaps to a slight extent, texture.

Humphries[1], in his pamphlet on the improvement of English wheat, defines another quality which bakers value, stability, the quality which makes large masses of flour easy to handle in the state of dough. This is an important point in the valuation of wheats, which I hope sometime to have the opportunity of investigating, but which is not touched upon in the present paper.

The best known suggestions for a chemical explanation of strength were mentioned by Humphries and Biffen in the paper already referred to, but it may be well, as this paper deals with the chemistry of the subject, to call attention to them again. The oldest idea suggested that strength was due to the gluten, which, in virtue of its tenacity, or perhaps viscosity, held in the bread the carbon dioxide produced by the yeast. No doubt a high content of gluten is frequently associated with strength, but there are so many cases in which a flour high in gluten is not so strong as another which contains less of that substance, that it can no longer be maintained that gluten content can be considered as a measure of strength. When the quantity of gluten failed to give the desired explanation, attention was turned to the quality of that substance, and it was examined both physically and chemically. The water-holding capacity of different glutens, as measured by the difference between the weight of the wet gluten immediately after extraction and its weight after drying, and the expansion of the gluten when heated to baking temperature in an instrument called the aleurometer, have both been credited with important bearings on the strength of the flour from which the sample of gluten was obtained. No generally accepted regularity seems to have been demonstrated. On the chemical side Girard and Fleurent[2] suggested that strength depends on the presence of a proper proportion of gliadin, which they fix at 75 per cent. of the gluten. Snyder[3] put forward the same suggestion, but fixed his ideal ratio of gliadin to glutenin at 65 per cent. of the former

[1] A. E. Humphries, *The Improvement of English Wheat*, published by the Millers' Association, 56, Mark Lane, E.C.

[2] *Le Froment et sa mouture*, Gauthier-Villars, Paris, 1903.

[3] Minnesota Expt. Stn., *Bulletin* 63, 1899.

to 35 per cent. of the latter. Many other investigators have worked on this branch of the subject, and their results are well summarised in what is practically a monograph on the baking value of wheat flours published last year by Dr F. F. Bruyning, Jr.[1], Director of the State Laboratory for Seed Control in Holland. Finally it has been suggested that it is not the ratio of gliadin to glutenin, but the absolute amount of gliadin in the flour which gives the flour its strength. So contradictory are the results of different workers that the theory that gliadin was the basis of strength has never been generally accepted, and the analyses of Hall[2] in the reports of the Home-grown Wheat Committee, and in his paper in the *Journal of the Board of Agriculture*, have conclusively shown that as often as not gliadin determinations fail completely to indicate whether a flour is weak or strong. In fact Hall[3], in his address to the Millers' Association, goes so far as to suggest that the name gliadin is little more than an "analytical label."

Reviewing the subject as it stands at the present time, it would appear that no one has succeeded in suggesting a generally satisfactory chemical or physical explanation of strength, and no one working on chemical or physical lines has used a definition of the term which does not include at least two distinct properties. Possibly these two facts may be cause and effect. The excellence of Humphries and Biffen's definition as a practical basis for the point they had in view is shown by the striking results Biffen has obtained in breeding new wheats which combine the vigour of the best English varieties with the strength of such foreign sorts as Fife, but even their definition includes two qualities, size and shape of loaf, which are quite likely to be chemically and physically entirely independent, and for each of which a separate explanation must consequently be sought.

Composition of Gliadin.

The peculiar physical properties of gliadin, as described by Osborne and Harris[4], suggest very strongly that it must have a considerable influence on strength, probably on the shape and texture components of that complex idea. As it had already been shown that neither the absolute percentage of gliadin in the flour, nor the ratio of gliadin to

[1] "La valeur boulangère du Froment," *Archives Teyler*, II. IX. 3, 4.

[2] *Journ. of Board of Agr.* XI. 1904, p. 321.

[3] *Report of Home-Grown Wheat Committee*, 1905–6.

[4] *Journ. Amer. Chem. Soc.* XXV. 1903, p. 343.

total proteid, gave satisfactory indications of strength, it seemed possible that as Hall suggested, gliadin might not be a definite substance, or that at any rate the gliadin of very strong flours might differ from that of very weak flours.

The composition of gliadin has been studied by Osborne and Harris, who have hydrolysed it with acids, and investigated its splitting products. They have also determined, by a modification of Hausmann's method, the percentages of amide-, diamino-, and monamino-nitrogen, which it contains. On reading their papers it appeared that their material had been made by extraction of several flours, all of which however must be classed as strong. It was possible therefore that some information might be obtained from a similar examination of gliadin made from a weak flour. Accordingly a quantity of gluten was washed out of two flours, *A* marked 95 for strength, and *B* marked only 40. The two samples of gluten, and also the two samples of flour, were extracted with 70 per cent. alcohol, and samples of gliadin made from each, and purified as described by Osborne and Harris. Approximately 1 gm. of each sample was then boiled for 8 hours with 30 c.cm. of strong hydrochloric acid, the resulting solutions evaporated to dryness, and steam distilled with magnesia into N/10 acid. The results are given below:

TABLE I.

Reference No.	Source of gliadin	Weight taken Grams	c.c. N/10 acid neutralized	Per cent. amide nitrogen in sample of gliadin	Per cent. total nitrogen in sample of gliadin	Per cent. amide nitrogen calculated to pure gliadin containing 17·66 per cent. total nitrogen	Per cent. amide nitrogen found in pure gliadin by Osborne and Harris
A	Flour	0·9780	29·0	4·16	16·74	4·40	4·30
	Gluten	0·9770	30·55	4·38	17·16	4·51	
B	Flour	0·9692	27·9	4·03	16·51	4·31	
	Gluten	0·9936	31·0	4·37	16·92	4·55	

The same problem was also attacked indirectly in the following way. Samples of gluten were washed out of six different flours, dried, and ground. Each was then hydrolysed, as in the case of the gliadin described above, and the percentage of amide nitrogen determined. The percentage of total nitrogen in the flours was determined by Kjeldahl's method, and the percentage of gliadin by extracting 16 gms. with 100 c.cm. of 70 per cent. alcohol, and determining the nitrogen in

20 c.cm. of the solution. The percentage of gliadin in the total nitrogen could then be calculated. The percentage of nitrogen in the gluten was also determined. Taking Osborne and Harris' figures, 17·6 per cent. total nitrogen in pure gluten, and 3·30 per cent. amide nitrogen in pure glutenin, and the mean of my own figures 4·44 per cent. of amide nitrogen in pure gliadin, the figures in the following table have been calculated:

TABLE II.

Source of gluten. Reference No. of flour	Bakers' marks of flour	Percentage in flour		Percentage gliadin nitrogen of total nitrogen	Percentage found in gluten samples		Percentage amide nitrogen in pure gluten calculated from	
		Total nitrogen	Gliadin nitrogen		Total nitrogen	Amide nitrogen	direct estimation	gliadin ratio in total proteid
D	95	1·69	1·01	60	13·0	3·10	4·19	4·08
E	80	2·44	1·23	50	12·1	2·69	3·91	3·87
H	80	2·19	1·10	50	10·5	2·40	4·02	3·87
G	(75)	2·32	1·15	49	10·7	2·45	4·02	3·86
F	63	1·34	·62	47	11·9	2·57	3·80	3·84
C	40	1·86	1·01	54	12·2	2·71	3·91	3·91

The results given in Table I. show that the amide nitrogen obtained by hydrolysing gliadins from typical strong and weak flours agree within the limits of error of the method, and the strong presumption therefore is that there is no difference in chemical composition between the gliadins of different flours. In the last two columns of Table II. the percentages of amide nitrogen in the gluten of six different flours as found by direct estimation are compared with the percentages calculated from the percentages of gliadin and glutenin in the different flours on the assumption that both these substances contain constant percentages of amide nitrogen. The figures so arrived at are found to agree as well as could possibly be expected, and there seems every probability therefore that the assumption as to the constant composition of the glutenin and gliadin of different flours is correct. The agreement would have been closer if the gliadin-glutenin ratio had been corrected for the small amount of proteid in the flours which is not either gliadin or glutenin. Such a correction would have slightly raised all the figures calculated from this ratio.

The second, third and fourth columns of Table II. clearly confirm

the conclusion of Hall and others already referred to, that neither the percentage of total nitrogen or of gliadin in the flour, nor the ratio of gliadin to glutenin, can be taken as indicating strength in flours which, like the above, were not grown under similar conditions.

Investigation of the nitrogenous compounds in flour, either from the point of view of their relative proportions, or from that of their chemical composition, does not seem likely to throw light on the meaning of strength, and it was therefore necessary to take up some other line of enquiry.

The Water-soluble Constituents of different Flours.

Having obtained nothing but negative results from the chemical examination of the gluten proteids, the next step was to turn to their physical properties. It is well known that the presence of exceedingly small amounts of salts, acids, or alkalis, may greatly modify the physical properties of proteids with which they are in contact. It seemed possible therefore that an examination of the water extracts of a number of flours of known baking properties might throw light on the question. For this purpose 100 grams of each of six flours were shaken for three hours with 1 litre of distilled water. The acidity of the extract was first measured by titrating 400 c.cm. with N/10 sodium hydrate solution in the presence of phenolphthalein. Using these proportions, the extract of highest acidity required 11·7 c.cm., that of lowest acidity only 4·4 c.cm. There was therefore a large difference in acidity, but these two extremes were found in flours having the same baking strengths. The intermediate acidities showed no relation to the strength of the flours from which they were made. These observations quite confirm those of Hall and others, and acidity does not appear to be related to strength.

Before beginning to estimate the soluble salts chemically I thought it would save trouble to get a rough idea of the total relative amounts of electrolytes in solution in the different extracts by determining their electrical resistances. Measurements were carried out, and indicated great differences in content of soluble salts, but no agreement with baking marks. For instance the highest resistances were given by the extracts of two flours marked respectively at 95 and 66, and the lowest by a flour marked at 80.

This was more or less what I expected, as the physical effect of salts on the proteid would probably depend rather on the ratio of salt to

proteid than on the absolute amount of the salt or salts, and from the rough measurements made there seemed to be some evidence of this ratio running parallel with the baking strength. The chemical examination of the soluble matter of the extracts was therefore proceeded with.

The method adopted was as follows: 100 c.cm. of each extract was evaporated to dryness, dried in the water-oven to constant weight, and weighed, to determine the total soluble solids. The residue was ignited, and weighed to determine the soluble ash. A duplicate set of determinations was then carried out with 500 c.cm. of extract. The ash of this larger quantity was dissolved in excess of N/10 acid, and back-titrated with N/10 alkali, using methyl orange as indicator, to determine the alkalinity of the ash, which was calculated as percentage of K_2O in the flour. Phosphoric acid was finally determined in the solution resulting from this last operation. Soluble nitrogen was determined in 50 c.cm. of extract by Kjeldahl's method. The results of this examination are tabulated below:

TABLE III.

Reference No. of flour extracted	Bakers' marks	Per cent. total nitrogen in flour	Percentages of soluble constituents of flours					
			Total solids	Ash	Nitrogen	Alkali as K_2O	Phosphoric acid as P_2O_5	Nitrogen- and ash-free extract
K	95	1·88	7·18	0·194	0·506	0·067	0·048	4·11
G	(75)	2·32	4·67	0·261	0·378	0·113	0·079	2·26
I	70	1·62	6·16	0·360	0·370	0·137	0·091	3·70
J	66	1·32	5·82	0·241	0·308	0·109	0·075	3·82
C	40	1·88	4·23	0·243	0·353	0·087	0·074	1·98

The figures given in Table III. show no regular agreement with bakers' marks, nor as already explained was this expected. In Table IV. the ratios of soluble ash, alkali, and phosphoric acid to total nitrogen in the flour are set out.

There seems to be a certain agreement between the ratios shown in Table IV. and the bakers' marks, the stronger flours containing more total nitrogen in proportion to their soluble salt content than the weaker ones, but to this regularity *G* and *C* are marked exceptions. Reverting to Table III., the last column, nitrogen- and ash-free extract,

or, in other words, the percentage of total soluble matter from which have been subtracted the percentage of ash and that of nitrogen multiplied by 5·7 to convert it into proteid, evidently runs parallel with bakers' marks, with the same exception of *G*, the bakers' mark for which is enclosed in brackets. This exception is extremely interesting.

TABLE IV.

Reference No. of flour	Bakers' marks	Ratios to total nitrogen of soluble		
		Ash	Alkali as K_2O	Phosphoric acid as P_2O_5
K	95	9·7	39	39
G	(75)	8·8	28	29
I	70	4·5	16	18
J	66	5·5	16	18
C	40	7·7	29	25

When I received it from Mr Humphries he wrote about its baking properties, "this flour will not make large loaves when baked by itself, but when blended with certain other flours behaves as if it possessed great strength." Here is an indication of two distinct factors, one possibly, one might almost say probably, governing the shape of the loaf, the other governing the volume. The first, on this supposition, would be the ratio of soluble salts to total proteid, or at any rate some factor which modifies the physical properties of the proteid, the second the nitrogen- and ash-free extract. *K* would then be strong because its proteid is in the presence of suitable conditions, and it also contains a high percentage of extract. *I* and *J* would be weak because they fulfil neither of these two conditions so well as *K*. In *G* the condition for good shape is fulfilled, but it cannot make large loaves, and cannot therefore on Humphries and Biffen's complex definition be accounted strong, because it lacks the volume factor, nitrogen- and ash-free extract. The same reasoning applies, though perhaps not so satisfactorily, to *C*. According to the figures in Table IV. it should on the score of ratios of salts to total proteid rank for shape of loaf above *I* and *J*, but it cannot do so in the bakehouse because it is low in the volume factor as shown by the extract figures in Table III.

This idea of separating strength into at least two factors, that of shape and that of volume, each independent of the other, at once seemed likely to be a good working hypothesis. As the volume factor obviously offered the simpler and more direct problem, I have mainly directed my attention to its investigation.

The Loaf-size Factor in Flours.

Soon after making the experiments which have just been described I received from Mr Humphries a sample of flour *L* made from the same kind of wheat as *G* which I had already tried, but of the 1906 crop. The report which accompanied the sample described it as "dingy in appearance, but behaving well in the bakehouse, and capable of making large loaves." Its total nitrogen and water-soluble constituents were at once determined as described above, and compared with those of the same wheat grown in 1905. The results of the comparison are given below:

TABLE V.

Reference No. of flour	Per cent. total nitrogen	Ratio to total nitrogen of soluble			Per cent. nitrogen- and ash-free extract	Remarks
		Ash	Alkali	Phosphoric acid		
G (1905 crop)	2·32	8·8	28	29	2·26	Bakes small loaves
L (1906 crop)	1·90	7·3	23	27	5·22	Bakes large loaves of good shape

These figures are an excellent confirmation of the two factor idea of strength: the flour of the 1906 crop has ratios of salts to total proteids almost identical with those of the 1905 flour, but it also contains more than twice as much nitrogen- and ash-free extract and therefore makes large loaves.

This nitrogen- and ash-free extract seemed therefore to be clearly indicated as the factor governing the volume of the loaf. As already stated it was determined by subtracting from the total soluble solids the sum of the ash and the soluble nitrogen multiplied by 5·7 to convert it into proteid. It is therefore the total soluble matter other than proteids. As to its composition, it is undoubtedly chiefly sugar

of one kind or other, probably mixed with various dextrins or dextrin-like substances. Jago[1] quotes many analyses of wheats and flours showing the presence of sugars, and expressly states that the sugar in the flour as such, together with that formed by diastatic action in the dough, is the source from which the yeast makes the carbon dioxide it produces in the process of fermentation. He does not seem to consider however that the percentage of sugar in any way influences the baking properties, unless for the worse, for he says that high sugar content in a flour indicates unsoundness. Girard and Fleurent[2] have also noticed great variations in the amount of sugar in flours. They state that in samples they have analysed glucose and cane sugar were present, the former varying from 0·09 to 0·81 per cent., and the latter from 0·63 to 1·89 per cent. Balland too[3] finds quite similar variations. Bruyning[4] considers that the sugar present in flours is not a mixture of glucose and cane sugar, but is in great part, if not entirely, maltose. Other investigators find cane sugar in the embryo of wheat, but not in the endosperm. The presence of raffinose in flour has also been mentioned. Halenke and Mosslinger, quoted both by Bruyning and by Konig[5], suggest that the baking value of a flour may be determined by digesting 2 gms. with water for two hours at 60° to 70° C., finally raising the temperature to 100° C., and filtering. Maltose is determined in the filtrate. In good flours they state that the maltose so determined amounts to 10—20 per cent., in bad ones to as much as 40—50 per cent. Their method appears to be rather drastic, and the sugar estimation to be difficult in the presence of all the dissolved starch. It calls attention however to the diastatic power of flours.

Evidently it is well known that flours contain a certain amount of sugar, whether glucose, maltose, or cane sugar does not matter for my purpose, for all are fermentable by yeast. It is also well known that flours in virtue of their diastatic power are able to form more sugar from the starch which they contain so abundantly, and that the sugar so formed, together with that originally present, forms the source from which the yeast makes the carbon dioxide it produces when dough is fermented. Since the size of a loaf depends on the expansion of the dough by the formation inside it of carbon dioxide in the process

[1] *The Science and Art of Bread Making.*

[2] *Le Froment et sa Mouture.*

[3] Quoted from Bruyning's Monograph.

[4] Monograph in *Archives Teyler.*

[5] *Landwirtschaftlich und gewerblich wichtiger Stoffe*, Paul Parey, Berlin, 1898.

of fermentation, or proving as it is called, and since the source of this carbon dioxide is sugar, the chief constituent of the nitrogen- and ash-free extract, the hypothesis that the proportion of this extract in flour influenced the size of the loaf seemed reasonable, and worth working out carefully.

The determination of the extract as described above involves three estimations, and the amount is then only arrived at by difference. An easier and more accurate method of arriving at the required result would be to make determinations of the sugar in the flour after incubation with water under conditions comparable with those to which dough is subjected in the process of baking. The easiest method of all however seemed to be to grow yeast in a mixture of flour and water, and measure the volume of carbon dioxide evolved. After some preliminary experiments this method was adopted, and when carried out as follows found to be quite satisfactory.

Twenty grams of the flour are weighed out into a wide-mouthed bottle, and a suspension of half a gram of yeast in 20 c.cm. of distilled water added and thoroughly mixed with the flour by stirring with a glass rod. The bottle is then closed with a rubber cork through which passes a delivery tube. The gas evolved is collected in a measuring tube, the bottle meanwhile being incubated in a water-bath kept at 35° C.

A convenient arrangement, if many flours have to be tested, is a large water-bath with clips soldered round it to hold a number of bottles, each of which can be connected by a rubber tube to a measuring tube. The differences observed in the volume of gas given off by various flours are so large that the apparatus described is quite accurate enough to measure them satisfactorily. The actual volumes of gas given off in the experiments varied from 345 c.cm. to 131 c.cm.

After a number of preliminary trials of the method with two flours at a time, a large apparatus was made, and the following series tested all at once.

Table VI.

Comparison of Bakers' Marks, Volume of Carbon Dioxide, and Size of Loaf.

Reference No. of flour	Bakers' marks	Volume of CO_2 evolved (S = 100)	Percentage total sugar in flour calculated as glucose	Increase in sugar after incubating 3 hours with water at 40° C.	Volume of loaf made from 100 gms. flour (S = 100)
S	85	100	2·3	2·7	100
P	90	94	2·6	0·6	85
O	96	90	—	—	80
L	85	88	2·5	—	88
T	(20)	83	1·8	0·4	81
M	73	79	2·0	0·6	78
N	(96)	77	2·2	0·4	—
Q	68	66	2·5	0·5	—
G 2	(85)	64	—	—	76
G 3	(85)	62	—	—	76
R	65	60	1·9	0·3	70
J	63	59	—	—	60
C	40	48	—	—	—
V	45	45	1·7	0·1	—
U 2	60	45	—	—	67
G	(75)	44	1·6	—	—
U	36	37	1·6	0·4	—

Inspection of the figures in Table VI., and of the curves in Fig. 1, shows at once that there is a general relation between bakers' marks and the volume of carbon dioxide which a flour can give off when incubated with yeast and water. It is clear however that there are several very marked exceptions, for instance, flours *T*, *N*, and *G*. The remaining flours show quite as close an agreement between bakers' marks and volume of gas as could be expected when it is remembered that bakers' marks are given for both size and shape of loaf, whilst gas evolution only deals with size.

The three exceptions were carefully investigated, and the result of the enquiry has given a most interesting confirmation to the idea.

G is the flour already mentioned which could not make large loaves when baked by itself, but when blended with certain other flours behaved as if it possessed great strength. It evidently could not make large loaves because it contained very little sugar, and consequently could give off very little gas. In describing it Mr Humphries wrote, "it seems to lack the something which makes the bread rise." I was

unable to obtain the identical flour with which it blended most successfully, but sample *S* may be taken as the same flour of the 1906 crop. I have also obtained flour made from the same variety of wheat grown

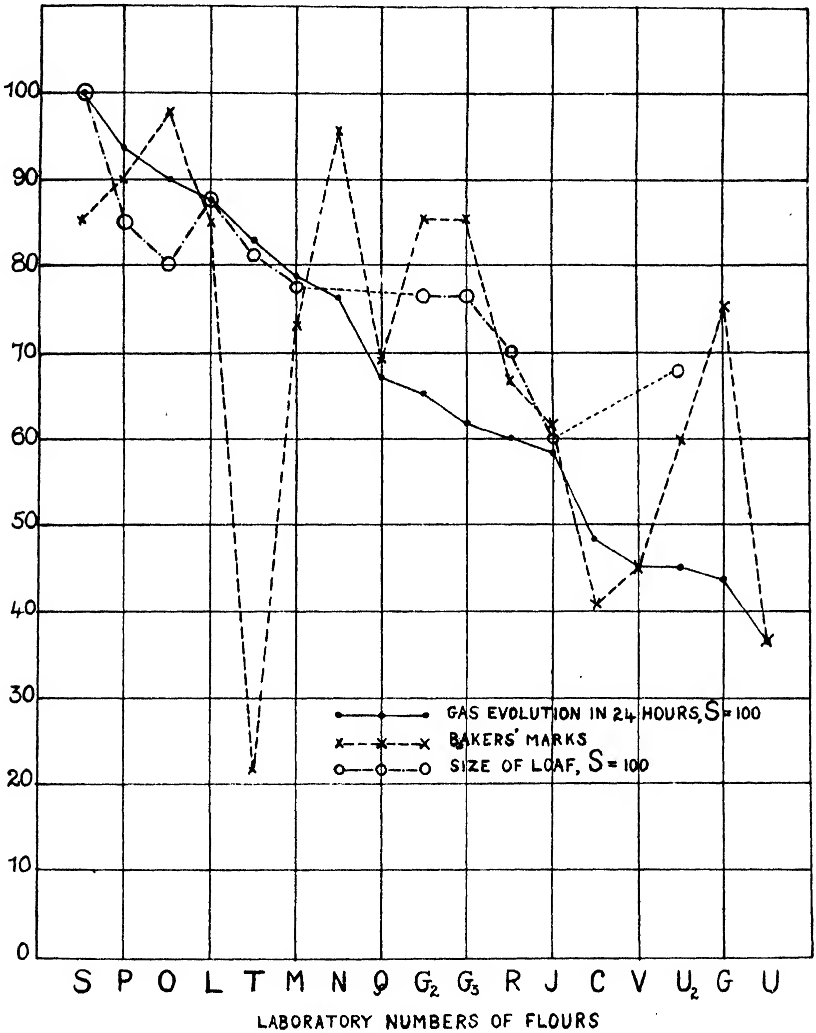


Fig. 1.

in 1905 in another district. Both these flours contained high percentages of sugar, both possessed high diastatic capacities, and both gave off large volumes of carbon dioxide when incubated with yeast.

There seems to be no difficulty therefore in explaining why *G* is an exception. It was evidently marked high on account of its capacity to act as a strong flour in blends, the other flour supplying the sugar in which it is lacking. The two factor idea of strength ought to be of the greatest use in deciding what flours to blend together. The flour *G* was found to give a large loaf when baked with the addition of sugar (Table IX.).

N like *G* is marked much higher than its gas evolution would indicate. On looking up the report which accompanied its marks, I found that it had been baked for marking with the addition of malt extract to the sponge. This would of course increase the diastatic capacity of the flour, and render more sugar available for the yeast, thus supplying the requisite gas evolution for making a large loaf. Unfortunately I had not sufficient material to bake this flour with and without malt extract, and thus definitely settle the point, but it seems quite clear that the high marking was the result of the artificial increase in the volume factor (sugar) by the addition of malt extract, and this exception is therefore brought into line. It is interesting here to note that it appears to be a common practice with bakers to add malt extract to certain flours, usually strong flours in which the shape factor, whatever that may be, is well developed, and thus to increase the size of the loaf. I believe sugar is also frequently used for the same purpose.

T was found on enquiry to be a most interesting case. When I found its gas evolution was altogether too high for its bakers' marks, it at once occurred to me that it might have been kept some time since the baking test was made, during which time it had undergone a large increase in strength, as noticed in the case of the extremely weak flour of some of the Rothamsted permanent wheat plots. I therefore asked Mr Humphries to repeat the test if he thought it likely that such might be the case. He very kindly did so, and reported that in the two months which had elapsed since the last test it had appreciated in strength to a mark of 40. He remarked that it made fairly large loaves of very bad shape. I was fortunately able to obtain more flour, and to bake it myself side by side with a number of others. On measuring the volumes by displacement of small-shot, *T* was found to occupy a place on the list quite in accordance with its relative gas evolution, and this last large exception is thus accounted for, partly by increase of strength on keeping, and partly by actually measuring the volume of the loaf and thus eliminating the shape factor from the

bakers' marking. My own baking experiments were made on a very small scale, but Mr Humphries very kindly repeated them for me in his bakehouse under my own supervision, and allowed me to measure the volume of the loaves. A two-pound loaf of four different flours on the average of two experiments measured: *T* 2294 c.cm., *M* 2281 c.cm., *R* 2086 c.cm., *J* 2012 c.cm. These figures entirely confirm my own small tests, and make the volume of the loaf follow the gas evolution. Very accurate agreement between gas evolution and size of loaf cannot be expected, for two obvious reasons: baking experiments cannot be carried out with any very great accuracy, since the temperature of the oven will vary from one place to another, and all the doughs cannot be put into the oven at what the bakers call the same stage of ripeness, and again the shape factor, whatever it may be, must influence the size of the loaf to a certain extent. The figures for gas evolution and size of loaf in Table VI. and in Fig. 1 follow one another as closely as can be expected, and seem to justify the conclusion that the capacity of a flour for giving off gas when incubated with yeast and water is the factor which in the first instance determines the size of the loaf.

So far as I am aware this factor has not been suggested as one of the components of strength by any investigator. That the idea must have occurred to Maurizio[1] is evident from perusal of an important paper published by him in 1902. In this paper Maurizio describes his investigations of the cause of the variations in the size of the loaf. He apparently did not attempt to test the capacities of the different flours he used to give off carbon dioxide when incubated with yeast and water. His idea was that the size of the loaf might depend on the fermentative capacity of the yeast. He baked loaves with yeasts of varying fermentative capacities, and found that he could establish no relation between the activity of the yeast and the size of the loaf. This is not surprising, as any yeast would produce abundance of gas during the long process of doughing if the flour provided it with enough sugar. In the second part of his paper, a series of experiments is described which bears more nearly on the point in question. These experiments were carried out as follows:—six flours were doughed with appropriate quantities of yeast and water, sugar being added to some in such amount that the volume of carbon dioxide given off in two hours at 30° C. was the same for each flour. These volumes were determined by calculation from the loss in weight of the doughs. The

[1] *Landwirtschaftliche Jahrbücher*, XXXI. 1902.

volume of the dough made without yeast was determined by measurement, and this volume added to the volume of carbon dioxide was taken as the theoretical volume of the dough. The actual volume to which the dough expanded in the process of rising was also measured, and compared with the theoretical volume. The theoretical volumes were approximately equal, varying only from 778 c.cm. to 705 c.cm., a difference of 13 per cent. The actual volumes of the dough varied from 450 c.cm. to 365 c.cm., a difference of 20 per cent. His next step was to calculate a theoretical volume for the bread, from the volume of the carbon dioxide expanded to the temperature of the oven, 250° C., and the volume of the solids of the bread. This is compared with the actual volumes of the loaves and the following variations are found: theoretical volumes 930 c.cm. to 840 c.cm., variation 11 per cent.: actual volumes 502 c.cm. to 290 c.cm., variation 73 per cent.

From these figures Maurizio concludes that gas escapes in the processes of doughing, rising and baking, and that the size of the loaf is therefore not proportional to the volume of gas evolved. These conclusions are obvious from my own figures. For instance I find that in 4 hours, the average duration of my baking experiments from the first mixing of the flour with yeast and water to the end of the baking, Fife flour will give off about 200 c.cm. of gas per 20 grams of flour. 100 grams would therefore give off 1000 c.cm. The size of the loaf baked from 100 grams of this flour is only 320 c.cm. There is evidently therefore a loss of gas amounting to about two-thirds of all the gas produced. Again I find that the volume of gas given off by 20 grams of different flours varies from 345 c.cm. to 131 c.cm., a variation of 163 per cent., as compared with a variation in the size of the loaf from 225 c.cm. to 376 c.cm., or only 67 per cent. There can, therefore, be no doubt either that loss of gas takes place both from the dough and in the oven, or that the size of the loaf is not proportional to the gas evolution, but these facts are quite consistent with the idea that the gas evolution has an effect on the size of the loaf. In his paper Maurizio gives another series of baking tests with the same flours, no sugar being used, and the yeast having about the same fermentative capacity as in the experiments already quoted. The results of the two experiments are tabulated below:—

Table VII.

Maurizio's Experiments, with and without sugar.

Flour No.	"Gärkraft" of yeast		Weight of flour in grams	Volume of loaf in c.cm.	
	No sugar experiment	Sugar experiment		No sugar	Sugar added to give off same volume of gas in all cases
1	79·26	85·9	100	458	502
2	,,	,,	,,	425	483
3	,,	,,	,,	419	463
4	,,	,,	,,	398	414
5	,,	,,	,,	311	334
6	,,	,,	,,	251	290
Difference per cent. between smallest and largest loaf				84	73

The figures show quite clearly that by adding sugar to the flour all flours cannot be made to produce equally large loaves, but they also show that the sugar has had an appreciable effect on the size, for without sugar the smallest and the largest loaves differ by 84 per cent. in size, whilst with added sugar the difference is reduced to 73 per cent.

Another important point is that Maurizio by adding sugar adjusted the conditions so that all his doughs gave off the same weight of gas in 2 hours. In this connexion the figures showing rates of gas evolution from 20 grams of flour, 20 c.cm. of water and 0·5 gram of yeast, in my own experiments, are of interest. These figures are set out in Table VIII.

Table VIII.

Rate of evolution of gas in c.cm. per hour by different flours when incubated with yeast and water.

Reference No. of flour	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	7th–24th hour
P	44	64	46	35	23	16	5·2
T	44	67	42	22	15	11	4·7
M	54	71	36	17	10	8	4·2
N	47	70	37	17	13	11	4·2
Q	50	60	25	14	13	11	3·0
R	46	54	22	15	12	9	2·7
V	42	39	18	11	9	6	1·9
U	45	34	11	8	6	4	1·3

It is at once evident on examining the figures that all the flours used give off gas rapidly when first mixed with yeast and water, and this rapid evolution lasts for about two hours. During this first period the yeast is presumably using up the sugar which existed as such in the flour. In Maurizio's sugar experiments his added sugar would increase the original rate of gas evolution, but all the sugar would be used up in the two hours' incubation, and when he moulded his loaves and prepared them for the oven, his added sugar would have been

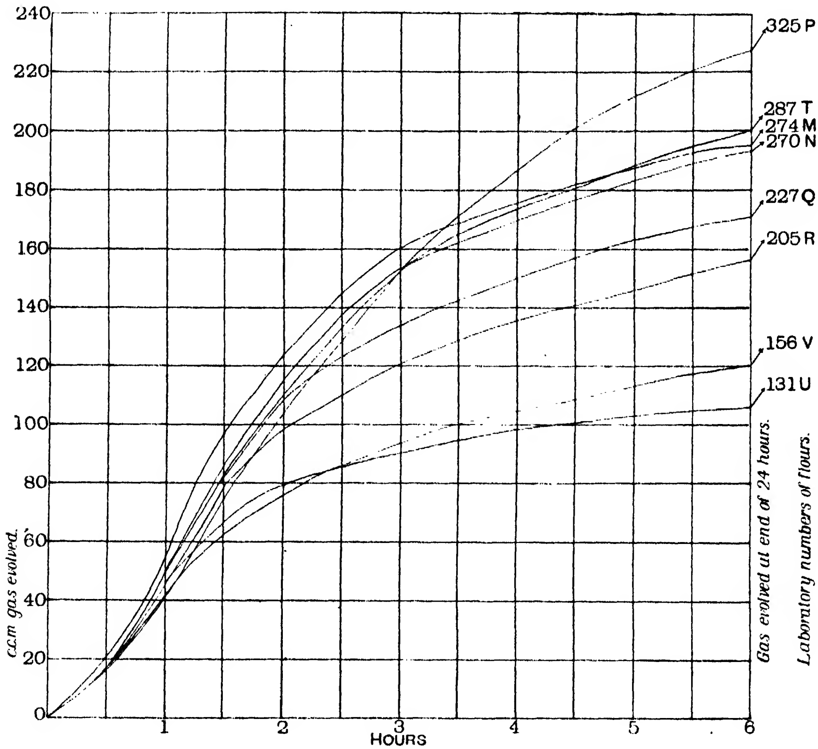


Fig. 2.

used up and the gas produced from it lost. This would certainly be so in ordinary practical baking. The gas which influences the size of the loaf is that which is given off during the later stages of fermentation, when the loaves have been moulded and are waiting to go into the oven. The figures in Table VIII. show clearly that in the case of the four flours *P*, *T*, *M*, and *N*, which make large loaves, gas evolution continues to take place at a sufficient rate to keep up a pressure in the

dough, and counterbalance the leak, even after the sixth hour of fermentation. This gas produced in the later stages of fermentation comes rather from sugar formed by diastatic action than from added sugar, or sugar originally present. In the flours *Q*, *R*, *V* and *U*, which make small loaves, the rate of fermentation becomes very slow in the later stages, so slow that it does not suffice to keep up a pressure in the dough, and the dough therefore becomes flabby, and cannot expand when put into the oven. If Maurizio had added sugar later in the fermentation he would probably have obtained different results, as is shown in the following experiments. These experiments were designed to test the idea that gas-forming capacity is the first factor in determining the size of the loaf. They were carried out as follows: two equal quantities of the same flour were weighed out into porcelain dishes, and warmed to 20° C. An appropriate quantity of water, warmed to 40° C., was measured out, and a weighed quantity of yeast

TABLE IX.

Effect of Added Sugar on the Size of the Loaf.

No. of experiment	Kind of flour used	Weight of flour in loaf	Per cent. sugar added	Volume of loaf without sugar	Volume of loaf with sugar	Increased volume due to sugar	Increased volume per cent.	Rate of gas evolution in c.cm. per minute at time of baking
		grms.		c.cm.	c.cm.	c.cm.		
1[1]	U	56	1	162	170	8	5	—
2[1]	,,	56	2	162	180	18	11	—
3[1]	,,	56	2	162	190	28	17	—
4[1]	,,	56	2	152	176	24	15	—
5[1]	,,	50	2	142	141	0	0	—
6[1]	,,	280	2	955	1018	63	7	{ 0·6 2·1
7[2]	Household	280	1	985	1035	50	5	—
8[2]	,,	280	1	995	1127	132	13	{ 1·7 2·1
9	G	100	5	250	350	100	40	—

[1] Average of three experiments. [2] Average of two experiments.

suspended in it. This yeast suspension was then poured into a depression in the flour, and about one-third of the flour stirred into it so as to make a thick cream, or sponge, as it is called by the baker. This was then incubated at 30° C. for about one hour. At the end of this incubation a weighed quantity of sugar was added to one of the sponges, both were then worked up into dough, and again incubated for

about one hour, when each was moulded into a loaf, put into a tall cylindrical tin, incubated for about half-an-hour, and finally baked in a gas oven at 250° C. During the whole process the two were treated in exactly the same way, except that one had sugar added to it, while the other had not. Tall tins were used to eliminate the shape question as far as possible, and to facilitate measurement of the volume of the loaves.

The figures in the above table show clearly that it is possible to increase the size of loaf which can be produced from flours of deficient gas-producing capacity by the addition of sugar in the later stages of the dough fermentation, a result which confirms the suggestion that the small size of the loaf in such flours is due to lack of gas evolution in the dough. The large increase in the case of the flour *G* is especially interesting in this connexion, as it is the flour of which the 1905 crop fell short of its reputation for strength, in that it could not make a large loaf because of its low sugar content and diastatic capacity (cf. Table V.).

In experiments 6 and 8 the rate of gas evolution at the time of baking was measured in the following manner: 600 grams of flour were weighed out and treated as already described. When the loaves were moulded, portions of dough corresponding to 280 grams of flour were weighed out for each loaf, and a second portion of each corresponding to 20 grams of flour was then put into a bottle connected with a measuring tube, so that the rate of gas evolution could be measured. The bottle was incubated side by side with the moulded loaves while they were rising, or proving as it is called, for baking. In experiment 6 the dough to which sugar had been added was found at the time of baking to be giving off gas at the rate of 2·1 c.cm. per minute, as compared with 0·6 c.cm. in the case of the same dough without sugar. In experiment 8 the corresponding rates were 2·1 and 1·7 c.cm. per minute. In both cases therefore increased rate of gas evolution was associated with increased size of loaf.

Similar measurements were also made in the case of several different flours. The results are given below:

Flour used	S	T	M	U
Relative rate of gas evolution at time of baking	2·0	0·6	0·4	0·2
Relative size of loaf	100	68	69	51

Here too the rate of gas evolution and the size of the loaf run parallel, and it seems certain therefore that it is more particularly the gas given off in the later stages of dough fermentation that determines the size of the loaf. This being so the size of the loaf will depend, not so much on the sugar present in the flour as such, as on the diastatic capacity, which will cause continued sugar formation, and consequently continued gas evolution in the dough. Probably therefore measurement of the gas evolved in the later stages of the fermentation would give a more accurate test for the power of making a large loaf than the measurement which I have made of the total volume given off in 24 hours. This idea corresponds with the view of Hays and Boss[1] who used the "second rise" as a test of the baking value of wheat flours in their breeding experiments. This point is under further investigation.

One further confirmation occurred to me. If the size of the loaf depends on the volume of gas evolved in the dough, then by using a constant weight of baking powder to provide the gas, instead of yeast, all flours should make loaves of approximately the same size. A number of small loaves were baked of flours which with yeast produce loaves of very varying sizes. Figures for these are given in the following table:

Laboratory No. of flour	Size of loaf. S = 100	
	Yeast	Baking powder
S	100	100
P	85	106
U	67	107

The experiment is satisfactory as far as it goes, that is to say, in the three cases tested the loaves differ greatly in size when baked with yeast, whilst with baking powder they give loaves which are sensibly of the same volume. It is however difficult to bake presentable loaves with baking powder, and I do not place much reliance on the figures obtained.

In conclusion I have great pleasure in again acknowledging my indebtedness to Mr A. E. Humphries for material and advice, and for kindly permitting me to carry out experiments in his bakehouse. I also have to thank my colleague, Mr R. H. Biffen, for much in-

[1] Minnesota Agric. Expt. Stn., *Bulletin* 62.

formation on the subject of wheats and flours, and Mr W. B. Hardy for advice on the physical properties of proteids and many other points. Messrs F. W. Foreman, G. Evans, and G. G. Chapman have from time to time given me valuable assistance in analytical work.

Summary.

Attention is drawn to the complexity of the ideas comprised in the term strength as applied to flour, and the necessity of investigating each idea separately.

The chemical composition of the gliadin and glutenin of strong and weak flours has been investigated, and it is shown that they are identical in all the flours examined. It is suggested therefore that the difference between strong and weak flours is connected rather with the physical properties of their gluten than with the chemical composition. Since it is well known that the physical properties of proteids are profoundly affected by small quantities of acids, alkalis, and salts, the amounts of these substances in strong and weak flours were determined. In the few cases examined, it was found that strength was associated with a high ratio of proteid to salts, and weakness with a low ratio. It is suggested that the variation of this ratio may be the explanation of the different physical behaviour of the gluten of strong and weak flours, and that this is the factor which determines that component of strength which governs the shape of the loaf, and its power of retaining gas. This point is receiving further investigation.

The factor which primarily determines the size of the loaf which a flour can make is quite distinct. The size of the loaf is shown to depend in the first instance on the amount of sugar contained in the flour together with that formed in the dough by diastatic action It is proposed to measure this by incubating the flour with yeast and water, and collecting the carbon dioxide evolved during 24 hours. Particular attention should be paid to the rate of gas evolution in the later stages of the fermentation, as this is shown to be more directly connected with the size of the loaf.

Taking Humphries and Biffen's definition of strength as "the capacity for making large well-piled loaves," and applying the above ideas, it is stated that the largeness of the loaf depends chiefly on the capacity of the flour to give off gas when fermented with yeast, especially in the later stages of dough fermentation, and the suggestion is made that shapeliness, and probably gas retention, are dependent on the physical properties of the gluten as modified by the presence of varying proportions of salts.

SOILS OF CAMBRIDGESHIRE.

By F. W. FOREMAN, Univ. Dipl. Agric.,
Cambridge University Department of Agriculture.

INTRODUCTORY NOTE[1].

In 1904, Sir James Blyth, Bart., a member of the Cambridge University Board of Agricultural Studies, offered a Scholarship tenable by a student who had taken the University Diploma in Agriculture, for the purpose of enabling him to spend a year in research. The offer was gratefully accepted, and the Scholarship was awarded to Mr F. W. Foreman. Mr Foreman submitted as a subject for investigation the Composition of certain of the Soils of Cambridgeshire. This subject was approved, and the work was carried out in the winter months of 1904—1905. The results are given in the following pages.

As I was partly responsible for the general plan of the work, I may explain the objects in view. In the first place, we were desirous of obtaining some general information about our local soils. Of recent years, the methods of mechanical analysis have been entirely recast, and it was obviously desirable that we should make use of these methods for the purpose of obtaining the fullest possible knowledge of the soils of the district. In the second place, we were anxious to ascertain whether there exists any close connexion between local soils and the underlying rock formation. On the subject of the relation of soil to rock, a good deal of work was done in the middle of last century, but for 50 years the question has received very little attention in Britain. Thus while both geologists and agricultural chemists have made great additions to their respective subjects, little that is new has been published in this country on the relations of Agriculture to Geology.

The Cambridge district is most interesting to the agricultural geologist. On the north-west and west of the county, and within a

[1] By Professor T. H. Middleton.

few miles of the town, one meets with stretches of Oxford, Ampthill, Kimeridge, Gault, and Boulder Clays; and also with the Lower Greensand, and Glacial and Post Glacial gravels; while the eastern half of the county contains the rich Chalk Marl, the Grey Chalk, the Middle and Upper Chalks, and a second stretch of Boulder Clay. The whole district is intersected by deep ditches and other surface sections, and the subsoils are easily studied. For reasons given in his paper, Mr Foreman did not investigate the whole of this area, but confined his work to the soils lying west of Cambridge.

In working out the relationship of soil and rock, the immediate purpose was to ascertain the accuracy of the information which an agriculturist could obtain from a geological map, and for this purpose, the clay formations in the area selected were particularly suitable. It is obvious that in the absence of surface deposits, a soil on a clay like the Gault must present a marked contrast to the soil on a sand like the Lower Greensand; but it is not so obvious that different clay formations will produce soils having distinctive features. It is not certain, for example, that a Kimeridge clay soil would more closely resemble a Kimeridge clay ten miles away than it would a Gault in the same neighbourhood. If well marked mechanical and chemical characters are to be found in any particular clay, then the geological map would be of great use to the agriculturist who for any reason might wish to obtain a knowledge of the general character of the soils of a district.

The one-inch drift map of the country round Cambridge is, unfortunately, rather old; and surface deposits have not received the same attention that is given to them in the maps now being published by the Geological Survey. Dr Teall, Director of the Survey, kindly offered to place the information in his office at our disposal, but for the preliminary work described in this paper, we did not find it necessary to make use of the six-inch maps. Mr W. G. Fearnsides, M.A., of Sidney Sussex College, gave us the benefit of his local knowledge, and thus enabled us to find typical samples, in different parts of the district, of those soils of which we were in search.

The samples of Boulder Clay were collected in a strip of country about 8 miles long between Haddenham and Hatley; the Gault samples were taken between Landbeach and Harlton, which are ten miles apart; the Greensand samples were taken between Aldreth and Potton, 20 miles apart; the Kimeridge Clays between Haddenham and Lolworth, 10 miles apart; and the Oxford Clays between Abbotsley and St Neots, 4 miles apart. In the last case, samples from other

districts would have been desirable, but in the time at his disposal, Mr Foreman could not deal with a greater number of soils than those described in his paper.

It may be objected that our selected samples representing typical Oxford Clay etc. represent at the same time a condition of things which could not occur in practice, as no surveyor would make a note of all the trifling deviations from the type which might affect the character of the surface soil, for most of these deviations would be without geological importance. There is a good deal of force in this objection; but while it may be admitted that we can never expect the colours of a one-inch map to have a precise agricultural meaning, it is clear that unless we are prepared to sample every field, and thus obtain average figures, that is, unless we are prepared to make an independent soil survey, we must confine our sampling to the type for which the colour stands.

In tracing the relationship between soils, Mr Foreman has made use of four sets of features: (1) the mechanical composition, (2) the chemical composition, (3) the common weeds, (4) the agricultural systems. The two last have been introduced chiefly to supply some facts of general interest about the soils; and the paper is more particularly concerned with laboratory characters. The question we ask is: "Does mechanical or chemical analysis show that any close relationship exists between the soils situated on the same formations?" Taking first the mechanical analyses, we find that they clearly disclose the wide differences existing between the clays and the sands. On the other hand, they sometimes show that very considerable variations occur between soils on the same formation. In the Greensands for example, the clay varies from 2·6 per cent. in soil No. 7 to 12 per cent. in soil No. 16; while in the subsoil of No. 12 (Kimeridge Clay) we have 42·6 per cent. of clay, and in No. 15 26·5 per cent. only. While these variations indicate the necessity for further figures before making general deductions from the analyses, the majority of the mechanical analyses do show a relationship between all the soils on the one formation. All the Gaults for example contain distinctly more fine silt and clay in the surface soil than do the Kimeridge Clays; and with the mechanical analysis of typical Gault before one, it would be impossible to mistake soils Nos. 1, 2, and 4, for Gault although they rest upon this formation, and to the casual observer closely resemble true Gault soils.

Apart from the proportions in which sand and clay are blended, the quality of soil depends chiefly on the quantities of lime, potash,

phosphoric acid, and nitrogen which are present. Of the first three, Mr Foreman gives the percentage found in the soils examined by him, and it is interesting to compare the clay soils in respect of these and other chemical characters. The Oxford is sharply marked off from the Kimeridge and Ampthill clays by the much higher percentage of lime present[1]. All the clays are abundantly supplied with potash, and contain very similar quantities of phosphates. The soils of the Gault contain more lime than those of the Oxford Clay, and containing little visible iron oxide, they differ much in appearance from the Kimeridge Clays.

Even with the limited material which Mr Foreman's work has placed at our disposal, we are justified in concluding that in the Cambridge area the soils are closely related in mechanical and chemical composition to the underlying formations, so that an agriculturist in possession of these analyses would find a good geological map of great use to him. From the agricultural standpoint, a good map is one which indicates the presence of any material that would much modify the character of the soil of any particular formation; such for example as lime, sand, or gravel on clay formations, or of clay on the surface of sandy beds. Mr Foreman's analyses afford illustrations of the kind of information required. Typical Kimeridge clay is very deficient in lime, but here and there thin bands of limestone occur, and when these mix with the soil, they entirely change its character, as is the case with soil No. 17. The agriculturist would therefore wish that the surveyor in mapping clays, should notice the smallest limestone deposits. Again, gravels have covered much of the Gault near Cambridge, and the presence of even small quantities in the surface have greatly modified the clay soils near Impington. See page 172. These gravels may have disappeared, and may only be traceable in pockets, or by pebbles in the surface soil, so that they may have no geological importance. But an indication of their presence on the map would put the agriculturist on his guard, and thus enhance the value of the map.

In drift-covered areas, the agriculturist cannot expect geological maps to have the same value to him as where drift is absent, but Mr Foreman's analyses show that in the case of the Boulder Clay lying west of Cambridge, the only notable difference between the soils is in the percentage of lime which is present; and the very different

[1] One Kimeridge clay does contain lime. For this there is an explanation. See page 173.

types found in soils Nos. 14 and 19 would at once be suggested if the map indicated that one Boulder Clay was chalky while the other was not. T. H. M.

THE DISTRICT INVESTIGATED.

In the neighbourhood of Cambridge the following strata are represented:

Recent Alluvium
River Gravels
Boulder Clay
Cretaceous :—
- Chalk { Upper, Middle, Lower
- Cambridge Greensand—(at one time dug for coprolites)
- Gault
- Lower Greensand

Jurassic :—
- Upper Oolites {Kimeridge Clay
- Middle Oolites {
  - Corallian {
    - Ampthill Clay = { Coral Rag, Coralline Oolite
    - Lower Calcareous Grit
  - Oxford Clay

In collecting soils from these formations some care is necessary. In many cases the one-inch drift map only serves as a general guide to the character of the surface soil. Gault for example may be mixed with Gravel or Boulder Clay, at the surface, or the soil of a Greensand may be mixed with Kimeridge Clay. The analyses given below, except Nos. 1, 2, 4, and 17, represent the composition of typical soils. For advice and assistance in selecting samples I am indebted to the kindness of Mr W. G. Fearnsides, M.A.

At the time of sampling notes were made upon aspect, elevation, drainage, the system of farming and the characteristic weeds. The agricultural information was usually obtained from the farmer himself.

The depth to which samples were taken varied slightly in different cases; the surface soil was sampled to its full depth, and the subsoil to a further depth of seven inches unless its character changed before this depth was reached.

The following is a list of the samples collected:

Reference No.	Formation	Parish	Survey No. of field or local name	Owner	Occupier
1	Gault	Impington	48	Mr J. Unwin	Mr J. Unwin
2	,,	,,	62	Mr J. T. Horrocks	Mr J. T. Horrocks
3	,,	Landbeach	'Old Hole'	Worts' Charity	Mr Hy. Cranfield
4	,,	Impington	39	Mrs W. Millar	Mr W. Millar
5	Lower Greensand	Gamlingay	—	Downing College	Mr Hy. Dew
6	Ampthill Clay	,,	140	Capt. Duncombe	Mr Plowman
7	Gault	Harlton	'Poundgate piece'	Christ's Hospital, London	Mr C. Whitechurch
8	,,	Madingley	79	Trinity College	Mr P. Papworth
9	Lower Greensand	Oakington	'Cottenham Field'	Mr J. H. H. Linton	Mr I. Cock
10	Oxford Clay	Abbotsley	67	Capt. Duncombe	Mr Wm. Rothery
11	,,	,,	101	,,	,,
12	Kimeridge Clay	Oakington	'Cuckoo Hill'	Mr J. H. H. Linton	Mr I. Cock
13	River Gravel	,,	'Peas Hill'	,,	,,
14	Boulder Clay	Hardwick	'Home Field'	Mr J. Hodson	Mr J. Hodson
15	Kimeridge Clay	Lolworth	2	Clare College	Mr J. Mitham
16	Lower Greensand	Oakington	173	Queens' College	Mr W. C. Cole
17	Kimeridge Clay	Haddenham	836	Mr P. A. S. Hickey	Mr A. Hepher
18	Lower Greensand	Aldreth	'Sandway'	Mrs Scott (Ely)	Mr I. Gay
19	Boulder Clay	Boxworth	163	Sir R. P. Cooper	Mr H. Eyre
20	,,	Bourn	'Red House Field'	Lord Clifden	Mr Jas. Broadway
21	,,	East Hatley	113	Downing College	Mr J. E. Ireland
22	Oxford Clay	St Neots	'Twenty-three acre'	Mr G. Fydell Rowley	Mr Rd. Aughton
23	Lower Greensand	Potton	175	Mr Geo. Smith	Mr J. Pibworth

METHODS OF ANALYSIS.

The method employed in making the mechanical analyses of the soils was that recommended by the Chemical Committee of the Agricultural Education Association[1].

Carbonates were estimated by the method devised by Amos[2].

The sizes of the particles aimed at in the mechanical analyses were as under:

TABLE I.

	Diameter in millimetres: maximum	Diameter in millimetres: minimum	Described as	
Stones and Gravel...	—	25	Stones	Separated by sifting
	25	10	Small Stones	
	10	3	Gravel	
Earth	3	1	Fine Gravel	
	1	·2	Coarse Sand	
	·200	·040	Fine Sand	Separated by subsidence
	·040	·010	Silt	
	·010	·002	Fine Silt	
	·002	—	Clay	

[1] *Journ. Agric. Sci.* Vol. I. Part 4, p. 470.

[2] *Journ. Agric. Sci.* Vol. I. p. 322.

In the chemical analyses the method of extraction employed was that of the Agricultural Education Association as described by A. D. Hall[1].

The phosphoric acid and potash were determined by the method of Neubauer[2] which was found to work very well.

It will be convenient to present the results of analyses in the following order:

Clay soils.	*Sandy soils.*
Boulder Clay	River Gravel
Gault	Lower Greensand
Kimeridge Clay	
Ampthill Clay	
Oxford Clay	

SOILS OF THE BOULDER CLAY.

Boulder Clay covers all the western side of the Cambridgeshire area "capping the high ground on the north of the Rhee valley and spreading in a sheet over the upland region between the valleys of the Ouse and the Cam; and further north a broad mass runs roughly from Kingston to Coton and Madingley, thence in a N.W. direction to Dry Drayton and Lolworth passing through Elsworth and Papworth into Huntingdonshire[3]."

The clay itself consists mainly of a dark grey or bluish mass weathering to a drab or brownish colour to the depth of several feet. The soil directly derived from it has a brownish colour and does not much differ in appearance from the underlying weathered mass. In the subsoil hydrated oxide of iron is visible. The amount of calcium carbonate in the soil and subsoil is nearly the same and runs about 1 per cent.

The soils themselves are extremely tenacious. The season must be very favourable indeed if a satisfactory tilth is to be obtained, and the crops are apt to be mediocre in the best of weather. In the summer wide cracks develop which are ruinous to pastures. When not cultivated the land soon becomes covered over with thorns and it may frequently be seen in a derelict condition. Draining and liming are very beneficial but the cost often makes these improvements

[1] *The Analyst*, November, 1900.

[2] *Landw. Versuchsstat*, 1905, LXIII. 141—149.

[3] Reed, *Geology of Cambridgeshire*.

impossible, amounting to more than the land is worth. The poorest type of pasture is found on undrained Boulder Clay, the roots of the grasses extending only a few inches into the soil, and as the closeness of the texture prevents movements of any kind there is an inadequate supply of air, and a spongy accumulation of undecayed roots collects at the surface.

The following table gives the mechanical analyses of the Boulder Clay soils, all of which were uniform in appearance.

TABLE II.

Mechanical Analysis of Boulder Clay Soils.

	No. 14 Boulder Clay above Gault		No. 19 Boulder Clay above Greensand		No. 20 Boulder Clay above Gault		No. 21 Boulder Clay above Grey Chalk	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Depth of sample in inches	7	7	6	7	7	6	6½	6½
Stones, diam. over 3 mm....	2·0	2·4	1·8	1·1	5·0	15·2	2·1	5·3
Fine Gravel	·38	·40	·67	·63	·50	1·07	·86	1·52
Coarse Sand	10·67	10·52	14·79	14·19	14·97	14·03	17·67	16·93
Fine Sand	14·95	14·72	18·55	17·66	15·57	13·87	12·74	14·71
Silt	11·85	12·11	16·37	16·79	12·36	12·05	11·29	11·88
Fine Silt	13·53	13·99	12·35	13·13	12·88	12·18	13·23	12·22
Clay	26·95	29·07	22·28	25·41	28·71	33·00	25·15	28·05
Moisture	3·66	3·44	3·24	3·58	4·40	4·21	4·44	4·46
Loss on ignition (less CO_2)	9·69	8·51	7·73	6·51	7·78	6·58	10·93	7·31
Calcium Carbonate	5·71	4·29	·58	·61	1·35	·99	1·02	1·30
	97·39	97·05	96·56	98·51	98·52	97·98	97·33	98·38

It will be seen that in spite of the fact of a different formation underlying the Boulder Clay, in each case there is a fairly close agreement in mechanical composition. As geologists have pointed out, the Boulder Clay of this district has had the same derivation and the underlying strata do not much influence the composition.

The merging of the rock through the subsoil into the soil is reflected very clearly in the analyses, there being a similar preponderance of the smaller particles in the subsoils and *vice versâ* with the larger particles in the soils.

Chemical analyses of the surface soils were made with the following results:

TABLE III.

Chemical Composition of Boulder Clay Soils.

	No. 14	No. 19	No. 20	No. 21
Moisture	3·66	3·24	4·40	4·44
Organic Matter, etc.	9·69	7·73	7·78	10·93
Insoluble Siliceous Matter ...	63·87	73·62	70·02	68·01
Lime (CaO)	3·95	·78	1·22	1·325
Magnesia (MgO)	·285	·28	·35	·60
Phosphoric Acid (P_2O_5)	·14	·113	·107	·102
Potash (K_2O)	·948	·785	·994	·963

Here the agreement is more marked than in the mechanical analyses, and it will be observed that all are low in phosphoric acid, high in potash, and fairly high in magnesia.

For comparison with the mechanical and chemical analyses the following agricultural notes are tabulated:

TABLE IV.

Crops grown on Boulder Clay Soils.

No. 14	No. 19	No. 20	No. 21
A better soil than the others, containing more Chalk and Phosphoric Acid. *Crops.* 1900 Barley 1901 Clover 1902 Wheat 1903 Oats 1904 Wheat All fair crops except the last wheat crop which yielded only 20 bushels per acre	A very difficult soil to work. *Crops.* 1898 Mangolds and Vetches (in place of usual bare fallow) 1899 Barley 1900 Oats, manured, but moderate crop 1901 Clover, moderate crop 1902 Wheat, moderate crop 1903 Beans, fair crop 1904 Wheat, manured with 15 loads Farmyard but only 20 bushels per acre owing to bad sowing season	Very difficult to work. *Crops.* 1899 Bare fallow 1900 Barley, 20—24 bushels per acre 1901 Beans, manured with 14—15 loads Farmyard, 12—16 bushels per acre 1902 Wheat, 16—20 bushels per acre 1903 Clover, 1¼ tons hay per acre 1904 Wheat	A very poor pasture which was greatly improved by a dressing of 10 cwt. Basic Slag per acre. See *Journ. Agr. Sci.* I. 125.

The common weeds were:

TABLE V.

Weeds of Boulder Clay Soils.

Specific name	Common name	Remarks
Avena fatua	Wild Oat	Gives great trouble
Ranunculus repens	Kingcob	,, ,, ,,
Brassica sinapis	Charlock	Very abundant
Sonchus arvensis	Sowthistle	,, ,,
Carduus arvensis	Thistle	,, ,,
Carduus acaulis	Dwarf Thistle	,, ,,
Rumex crispus	Dock	,, ,,
Galium aparine	Goose grass	Plentiful
Agrostis stolonifera	Bent	Very troublesome
Daucus carota	Wild Carrot	Fairly numerous
Geranium molle	Dove's foot Geranium	
Plantago media	Plantains	
Veronica agrestis	Speedwell	
Taraxacum densleonis	Dandelion	
Tussilago farfara	Coltsfoot	
Stellaria media	Chickweed	In small quantity
Senecio vulgaris	Groundsel	,, ,,
Ononis arvensis	Rest Harrow	A scourge in the pastures

SOILS OF THE GAULT.

An irregular and fairly wide strip of Gault extends from the S.W. to the E. of the County of Cambridgeshire and thence into Norfolk to Stoke Ferry and beyond. Gault, on weathering, changes to a creamy colour, and gives rise to quite a light coloured soil. No hydrated ferric oxide can be seen. The soils are thus readily distinguished from those of the Kimeridge and Boulder Clays. In this district Gault soils contain a good deal of calcium carbonate which may vary from 3 to 10 per cent. The subsoil nearly always contains a layer of the small rounded chalk pebbles in various stages of disintegration, termed "race" by geologists. In every case a higher percentage of chalk is found in the subsoil after removal of the pebbles than in the soil. Gault soils are very stiff and sticky, but though containing more "clay" are not quite so tenacious as those of the Boulder Clay. The higher content of calcium carbonate modifies the physical properties considerably. Gault soils are very difficult to till, a bare fallow is absolutely necessary at frequent intervals. As a rule it is found however that fair crops can be obtained on these soils in favourable seasons if they are well cultivated.

Three of the Gaults (Nos. 1, 2 and 4) were among the first soils sampled, and as they proved not to be typical, the analyses are given separately. These soils represent a common type, where Gault is mixed with Gravel. They were taken in the neighbourhood of Impington.

The composition of typical Gault soils is shown in Table VI.

TABLE VI.

Mechanical Analyses of typical Gault Soils.

	No. 3		No. 7		No. 8	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Depth of sample in inches...	5½	6	5¾	6¾	6½	6¾
Stones, diam. over 3 mm....	per cent. 3·4	per cent. 9·0	per cent. 1·0	per cent. ·6	per cent. ·6	per cent. 1·0
Fine Gravel	1·18	1·17	·19	·05	·50	·38
Coarse Sand	18·53	13·01	4·33	1·16	9·87	8·21
Fine Sand........................	7·08	5·53	4·53	2·30	7·80	7·38
Silt	6·06	6·79	11·97	10·65	10·60	9·23
Fine Silt	13·89	14·68	21·27	18·22	15·57	14·62
Clay	33·04	40·36	31·68	39·96	34·64	38·38
Moisture	4·42	4·86	3·82	4·26	3·14	3·78
Loss on ignition (less CO_2)	10·52	8·28	10·83	7·45	10·51	9·15
Calcium Carbonate............	2·05	3·23	8·30	14·71	5·34	8·11
	96·77	97·91	96·92	98·76	97·97	99·24

The increase of the larger particles and decrease of the carbonate of lime as rock passes upwards into soil is again plainly shown in all.

Table VIII. shows the chemical composition of the typical Gault soils.

The percentages of lime and potash are high, while all three soils, and especially No. 8 (a worn-out pasture), are deficient in phosphates.

The agricultural notes relating to the typical Gault soils are given in Table IX.

The common weeds found are given in Table X.

Dandelion appears to be especially favoured by the Gault.

Table VII.

Mechanical Analyses of Gault mixed with Gravel.

	No. 1		No. 4		No. 2	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Depth of sample in inches	5	5½	6	6½	4½	5½
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Stones, diam. over 3 mm.	1·5	1·5	1·1	1·3	4·4	3·9
Fine Gravel	·55	·80	1·13	·70	·88	1·48
Coarse Sand	9·43	11·75	12·97	11·70	33·37	26·88
Fine Sand	11·18	11·21	11·35	12·25	14·69	13·48
Silt	18·60	18·73	15·44	14·21	13·40	9·75
Fine Silt	13·63	11·61	13·39	13·13	8·78	7·77
Clay	18·46	20·03	18·43	20·79	14·12	23·50
Moisture	3·74	2·32	3·72	4·03	2·30	2·40
Loss on ignition (less CO_2)	8·92	6·44	8·29	8·32	8·57	7·46
Calcium Carbonate	10·98	11·40	10·28	9·91	1·21	5·22
	95·49	94·29	95·00	95·04	97·32	97·94

Table VIII.

Chemical Composition of Gault Soils.

	No. 3	No. 7	No. 8
Moisture	4·42	3·82	3·14
Organic Matter, etc.	10·52	10·83	10·51
Insoluble Siliceous Matter	63·67	56·29	60·35
Lime (CaO)	3·87	7·28	4·74
Magnesia (MgO)	·29	·15	·215
Phosphoric Acid (P_2O_5)	·14	·127	·097
Potash (K_2O)	1·14	1·143	1·30

TABLE IX.

Crops grown on Gault Soils.

No. 3	No. 7	No. 8
Crops. 1901 Wheat 1902 Clover 1903 Barley 1904 Barley again, because owing to bad season wheat could not be sown. Steam cultivated but very light crop. Other crops fair.	1901 Wheat, fairly good crop 1902 Vetches, fairly good crop 1903 Oats, bad sowing-time and poor crop 1904 Wheat, moderate crop	Very worn-out pasture broken up and two crops of oats taken without manure; each yielded about 8 bushels per acre.

TABLE X.

Weeds of Gault Soils.

Specific name	Common name	Remarks
Taraxacum densleonis	Dandelion	Very abundant
Ranunculus repens	Kingcob	,, ,,
Tussilago farfara	Coltsfoot	,, ,,
Carduus arvensis	Thistle	,, ,,
Rumex crispus	Dock	,, ,,
Brassica sinapis	Charlock	,, ,,
Euphorbia peplus	Petty spurge	In smaller quantity
Geranium molle	Dove's foot Geranium	,, ,,
Veronica agrestis	Speedwell	,, ,,
Senecio vulgaris	Groundsel	,, ,,
Potentilla reptans	Cinquefoil	,, ,,
Stellaria media	Chickweed	,, ,,

SOILS OF THE KIMERIDGE AND AMPTHILL CLAYS.

The principal outcrop of the Kimeridge Clay is in the neighbourhood of Ely and Haddenham, a narrow band also runs from Knapwell through Boxworth, Oakington and Cottenham beyond which it spreads out beneath the Fenland of North Cambridgeshire. The Kimeridge Clay consists of dark blue or dark grey clays. Thin layers of limestone in continuous bands or as lines of septarian nodules are found in it, but the clay between these bands is absolutely devoid of chalk; crystals of selenite however are scattered throughout its mass. Except where these thin bands of limestone reach the surface, the soils derived from

TABLE XIV.

Weeds of the Kimeridge and Ampthill Clays.

Specific name	Common name	Remarks
Avena fatua	Wild Oat	Very troublesome
Tussilago farfara	Coltsfoot	,, ,,
Carduus arvensis	Thistle	,, ,,
Carduus acaulis	Dwarf Thistle	,, ,,
Rumex crispus	Dock	,, ,,
Brassica sinapis	Charlock	,, ,,
Stellaria media	Chickweed	Fairly prevalent
Taraxacum densleonis	Dandelion	,, ,,
Galium aparine	Goosegrass	,, ,,
Veronica agrestis	Speedwell	,, ,,
Geranium molle	Dove's foot Geranium	,, ,,

SOILS OF THE OXFORD CLAY.

The principal outcrop of this formation unobscured by Drift is to the west of the county of Cambridgeshire where it borders with Huntingdonshire. One of the samples (No. 22) came from St Neots,

TABLE XV.

Mechanical Analyses of Oxford Clay soils.

	No. 10		No. 11		No. 22	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Depth of sample in inches	6½	7	6	7	6¼	6½
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Stones, diam. over 3 mm....	1·3	1·7	3·0	1·6	3·4	1·8
Fine Gravel....................	·75	1·16	·85	1·16	1·17	·46
Coarse Sand....................	14·64	13·27	12·33	10·44	15·56	15·15
Fine Sand	7·92	7·91	8·63	7·81	12·86	9·75
Silt	11·67	11·51	14·81	14·24	11·80	7·56
Fine Silt	13·81	14·49	13·40	14·45	12·55	13·39
Clay	29·97	34·96	29·63	30·74	27·16	27·39
Moisture	3·24	2·46	3·25	2·10	4·18	3·72
Loss on ignition (less CO_2)	9·87	7·38	9·99	7·64	7·59	6·66
Calcium Carbonate............	3·04	6·63	4·72	8·90	4·46	11·27
	94·91	99·77	97·61	97·48	97·33	95·35

another (No. 11) from the experimental field of the Cambridge University Department of Agriculture at Abbotsley, which was laid down to pasture in 1900, and the third (No. 10) from an arable field in the vicinity.

In the district mentioned the Oxford Clay itself is a bluish grey clay weathering to a light colour, and the soil and subsoil are not unlike those of the Gault, perhaps a shade darker in colour. The subsoil contains a little hydrated ferric oxide which is just visible. The Clay is distinguished by containing crystals of selenite and nodules of pyrites. At the surface quite a quantity of a characteristic fossil (*Gryphaea dilatata*) is to be found. The subsoil contains small chalk pebbles resembling those in the subsoil of the Gault.

The soils are not quite so sticky as those of a typical Gault, but in all other respects they are much the same. Very little difference can be seen between the subsoil and soil, and the passage upward from rock to soil is not pronounced.

TABLE XVI.

Chemical Composition of Oxford Clay soils.

	No. 10	No. 11	No. 22
	per cent.	per cent.	per cent.
Moisture	3·24	3·25	4·18
Organic Matter, etc.	9·87	9·99	7·59
Insoluble Siliceous Matter	65·01	65·58	67·18
Total Lime (CaO)	3·23	3·22	3·08
Magnesia (MgO)	·215	·47	·25
Phosphoric Acid (P_2O_5)	·118	·145	·138
Potash (K_2O)	1·06	1·11	1·09

These soils resemble each other closely.

The experiments carried out on No. 11 showed basic slag to be a very profitable manure. The soils are intractable, unless dry, and bare fallowing is necessary every four or five years as couch (*Agropyrum repens*) otherwise becomes very abundant.

The following is a list of recent crops and yields:

TABLE XVII.

Crops grown on the Oxford Clay.

No. 10	No. 11	No. 22
1899 Clover, very fair crop 1900 Wheat ,, ,, 1901 Fallow 1902 Barley, very fair crop 1903 Beans ,, ,, 1904 Wheat, very bad crop, bad weather at seed-time	1896 Fallow 1897 Barley, good crop 1898 Beans ,, ,, 1899 Wheat ,, ,, 1900—1904 Pasture	1901 Summer fallow, permanent pasture seeds failed 1902 Wheat, 20 bus. per acre *1903 Oats, 56 bus. per acre 1904 Wheat, 20 bus. per acre (bad seed-time)

* Manured with 15—20 tons mixed London dung and farmyard manure per acre.

TABLE XVIII.

Weeds of the Oxford Clay.

Specific name	Common name	Remarks
Tussilago farfara	Coltsfoot	Very troublesome
Brassica sinapis	Charlock	,, ,,
Ranunculus repens	Kingcob	,, ,,
Carduus arvensis	Thistle	,, ,,
Rumex crispus	Dock	,, ,,
Geranium molle	Dove's foot Geranium	Abundant
Anthemis Cotula	Stinking Mayweed	,,
Sherardia arvensis	Field Madder	,,

SOIL DERIVED FROM RIVER GRAVEL.

It will be seen that this is quite a similar type of soil to those of the Lower Greensand. The sample was taken from a field adjoining a small watercourse. This was considered by the occupier to be very good land indeed. The recent crops were:

1901 Barley, good crop.
1902 Peas, good crop, had Farmyard manure.
1903 Wheat, very good indeed.
1904 Barley, moderate crop on account of dry season.

The weeds were very similar to those found on the Greensand soils with, in addition, a very large quantity of what is locally termed "Hardhack" (*Centaurea nigra*).

TABLE XIX.

Mechanical Analyses of River Gravel soil and subsoil.

	No. 13	
	Soil	Subsoil
Depth of sample in inches...	6½	6½
	per cent.	per cent.
Stones, diam. over 3 mm....	9·0	10·7
Fine Gravel....................	2·22	2·40
Coarse Sand....................	49·80	51·90
Fine Sand	18·14	16·81
Silt	7·20	7·64
Fine Silt	5·90	5·70
Clay	9·74	8·97
Moisture	1·00	·96
Loss on ignition (less CO_2)	5·20	5·12
Calcium Carbonate...........	·045	nil
	99·245	99·50

SOILS OF THE LOWER GREENSAND.

The principal outcrops of the Lower Greensand in Cambridgeshire are:

(1) Around Aldreth and Haddenham.

(2) A narrow strip running from Lolworth through Oakington to Cottenham.

(3) At Gamlingay and extending through Potton and Sandy into Bedfordshire.

The weathered Greensand is red because of the oxidation of the ferrous iron in the original glauconitic grains, and it is characterised by the "box stones" and iron stones it contains. These are ferruginous concretions. Greensand gives rise to red soils which are liable to be severely scorched in summer.

Here the larger sized particles are very abundant, as much as from 60 to over 80 per cent. were stopped by a sieve with 100 meshes to the linear inch. The percentage of "clay" (which is of a totally

TABLE XX.

Mechanical Analyses of Lower Greensand soils.

	No. 5		No. 23		No. 18		No. 9		No. 16	
	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
Depth of sample in inches	6¼	6¼	7	6	6	6½	7	6½	5½	6
Stones, diameter over 3 mm.	per cent. 5·3	per cent. 4·2	per cent. 5·7	per cent. ·9	per cent. ·8	per cent. ·3	per cent. 6·1	per cent. 10·2	per cent. 2·7	per cent. 3·8
Fine Gravel	2·62	1·73	7·15	6·57	1·57	1·84	3·25	4·93	3·03	2·7
Coarse Sand	71·90	73·93	81·78	77·65	67·93	70·63	54·54	47·16	55·78	52·81
Fine Sand	4·62	5·08	3·37	6·14	7·35	6·36	9·92	8·22	11·14	8·54
Silt	4·23	4·37	1·80	1·33	4·49	3·92	8·81	9·87	8·05	7·86
Fine Silt	2·21	2·01	·81	1·01	3·89	2·89	4·15	4·66	4·20	4·43
Clay	7·20	6·74	2·63	3·57	7·67	11·10	7·88	18·39	12·06	17·16
Moisture	1·24	1·77	·57	·74	1·70	1·22	1·40	1·92	1·56	2·16
Loss on ignition (less CO_2)	5·58	3·99	2·47	2·16	4·58	3·16	5·94	7·02	5·20	4·86
Calcium Carbonate	nil	nil	nil	nil	·318	·038	nil	nil	nil	nil
	99·60	99·62	100·58	99·17	99·498	101·158	95·89	102·17	101·02	100·52

different nature from that of the heavy soils, being red in colour and nearly all ferric oxide) is sometimes higher than would be expected. A noteworthy feature is the total absence of calcium carbonate. The soils are very dependent upon the addition of humic matter to increase their water-holding capacity. Those found in the Gamlingay and Potton district are coarser grained than those from the Lolworth and Oakington Greensand. In the latter some of the "clay" has been washed through into the subsoil, forming a hard pan when dry. Most of the samples were found to be acid in reaction. The soils around Gamlingay and Potton grow market garden produce. They are heavily manured with London dung and respond to liberal treatment. The soils near Lolworth and Oakington which contain a higher percentage of clay are naturally superior to those of Gamlingay. All the Greensand soils are easy to work, the great difficulty of the farmer being lack of moisture, but the liberal use of London dung or farmyard manure greatly increases their water-holding power.

TABLE XXI.

Chemical Composition of Greensand soils.

	No. 5	No. 23	No. 18	No. 9	No. 16
Moisture	1·77	·57	1·70	1·40	1·56
Organic Matter, etc.	5·58	2·47	4·58	5·94	5·20
Insoluble Siliceous Matter	82·03	91·22	85·13	80·94	80·97
Lime (CaO)	·065	·085	·34	·26	·15
Magnesia (MgO)	·08	·125	·24	·17	·14
Phosphoric Acid (P_2O_5)	·204	·259	·197	·169	·146
Potash (K_2O)	·236	·278	·47	·443	·47

It will be seen that these correspond very closely, revealing a fairly high content of phosphate, little potash, and very little magnesia and total lime.

The quality and cropping powers of these soils may be inferred from the following particulars.

TABLE XXII.

Crops grown on the Greensand.

No. 5	No. 23	No. 18	No. 9	No. 16
Fairly elevated situation. 1901 Potatoes, fair crop 1902 Oats " " 1903 Potatoes " " 1904 " " " Scorching in summer a difficulty.	Elevated situation and very liable to scorching. Land had been barren for 20 years, then— 1902 Potatoes, very bad crop 1903 Rye, 20 bus. per acre 1904 Peas, 12 bus. per acre 20 tons dung per acre used for Peas.	Very good soil. 1902 Clover, 1½ tons hay per acre 1903 Wheat 32 bus. per acre 1904 Swedes, a little finger and toe in patches 1905 Barley Soil suited to a damp season.	A very fertile field. 1902 Clover, 1½ tons hay 1st crop, 1 ton hay 2nd crop 1903 Barley, averaged 40 bus. per acre 1904 Barley, averaged 40 bus. per acre	Good soil. 1902 Wheat, 28 bus. per acre 1903 Swedes, good crop fed off by sheep 1904 Barley, 24 bus. per acre only, because of dry weather 1905 White Clover Beans used as an alternative to clover because of clover sickness.

TABLE XXIII.

Weeds of the Greensand.

Specific name	Common name	Remarks
Stellaria media	Chickweed	Very large quantities
Chenopodium album	Fat hen	Large quantities
Polygonum aviculare	Knot grass	" "
Capsella bursa pastoris	Shepherd's purse	" "
Senecio vulgaris	Groundsel	" "
Spergula arvensis	Field Spurrey	" "
Rumex acetosa	Sorrel	" "
Lychnis vespertina	Campion	" "
Papaver Rhoeas	Poppy	" "
Veronica agrestis	Speedwell	" "
Crysanthemum segetum	Wild Marigold	Especially where acid
Viola tricolor	Field pansy	Large quantities
Plantago lanceolata	Plantain	Fairly numerous
Anthemis Cotula	Stinking Mayweed	" "
Sherardia arvensis	Field Madder	" "
Daucus carota	Wild Carrot	" "

On No. 16, Crysanthemum segetum and sorrel grew almost exclusively on those patches where "Finger and Toe" attacked the swede crop. This was also the case on soil No. 9.

THE HYBRIDISATION OF BARLEYS.

By R. H. BIFFEN, M.A.,

Agricultural Department, Cambridge University.

THE following experiments on the hybridisation of barley were commenced in 1901; consequently the inheritance of some of the characteristics has been known for some time. In view of the fact that from the earlier accounts of Rimpau and Tschermak the main outlines of the story could be traced without any special difficulty, there appeared to be no necessity for immediate publication. This was delayed, therefore, in order to complete the investigation of one or two doubtful points. The literature of barley-breeding published prior to the year 1900 is rather extensive, but of comparatively little interest to the modern student of heredity. The most important papers are those of Liebscher[1] and Rimpau[2]. That part of Rimpau's great paper, "Kreuzungprodukte Landwirtschaftliche Kulturpflanzen," dealing with barley, is of exceptional interest on account of its extreme thoroughness and the completeness of the records it contains. As one reads it one cannot help thinking that Rimpau must have come very near to making an independent discovery of Mendel's laws of inheritance.

The details of the cross between Steudelgerste and Gabelgerste will serve to illustrate this point. In these varieties the following characters are represented, four and two rows of grain, awned and trifurcate paleae, black and white colour and naked and covered grain. Rimpau raised a large number of plants from the grain of the hybrids and found that they could be grouped into a series consisting of sixteen distinct types as follows: four or two row barleys with normal or trifurcate awns: black and white forms of each of these four types

[1] Liebscher, *Bot. Centrb.* Vol. XL. p. 232 (abstract).

[2] Rimpau, *Landw. Jahrb.* Vol. XX. p. 354. See also Wittmack, *Ber. d. deut. Bot. Ges.* IV. p. 434.

which finally had either naked or enclosed grain. These are the sixteen types expected on the assumption that the characters present in the parents are capable of independent segregation. Rimpau raised three further generations of all of these types, but evidently sowed grain from a number of individuals of each. Their behaviour is tabulated in some detail. One or two anomalous cases occur in the descriptions which are probably due more to self-sown grain than actual errors, but on the whole the analysis of their behaviour agrees well with the results expected from the Mendelian standpoint. Other cases are examined in the same fashion, but apparently on a less extensive scale, and these also show the same general correspondence with the expectations we should form to-day.

In the following experiments some of the types resulting from Rimpau's crosses have been used as parents together with many other varieties. For the majority of these I am indebted to Mr E. S. Beaven of Warminster, who kindly presented a large collection to the Cambridge Agricultural Department.

The numerous varieties of barley in existence provide a large number of differentiating characteristics. These are described below at some length because many are wanting in our cultivated types which form a very small proportion of those known, but not cultivated on an extensive scale.

The distinguishing characteristic of the genus Hordeum is that the one-flowered spikelets are arranged in groups of three alternately on the rachis. Each of the spikelets has a pair of glumes at its base. The cultivated barleys are grouped together as a single species *Hordeum sativum* (Pers.) divided into a number of sub-species. These probably represent elementary species as defined by de Vries. As in most large groups of closely related forms the classification is often a matter of considerable difficulty. Perhaps the most convenient scheme is that of Körnicke as modified by Beaven[1]. In this the following sub-species are recognised:—*H. hexastichum, H. vulgare, H. intermedium, H. distichum, H. zeocriton,* and *H. decipiens.*

H. hexastichum is sometimes grown in this country for forage, and is then winter sown. It is commonly found in commercial samples of Persian, Chilian, and Beyrout barleys. In this group all of the spikelets are fertile and the internodes are short. It is therefore known popularly as six row barley with dense or wide ears.

[1] Beaven, *Journ. Inst. of Brewing,* Vol. VIII. p. 542.

H. vulgare is commonly known as "bere" or as four rowed barley. Körnicke names the group *H. tetrastichum* on this account. In reality the barley is six rowed, so that the term is deceptive. Its use appears to be responsible for the idea that in this type the median floret of the group of three has been suppressed, leaving only pairs of lateral florets on either side of the rachis. It differs from *H. hexastichum* in having long internodes so that the ears are lax or narrow. The lateral florets of alternate groups partially overlap one another, and consequently the six rows are not so clearly marked as they are in *H. hexastichum*, and the ear appears to be more or less four-sided. Varieties of *H. vulgare* are largely imported into this country from California, the Argentine, Smyrna, and the Black Sea districts. "Bere" is also cultivated to a certain extent.

H. intermedium is a group consisting of at present only two varieties of no importance from the technical point of view, but of some interest to the biologist. The lateral florets in these are small and awnless, but as a rule they set grain. One of the varieties was found by Haxton in a field of bere, and he described it as a transition form between bere and the normal two rowed barley[1]. In my cultures many of the lateral florets are frequently sterile.

H. distichum has a fertile median floret and sterile laterals in each group of three spikelets. The lateral florets are staminate and reduced in size. The internodes are long, and consequently the ears are described technically as narrow and two rowed. The group is represented in this country by the Chevallier and Archer barleys.

H. zeocriton is characterised by staminate lateral and fertile median florets and a dense or wide ear. *H. distichum* and *H. zeocriton* are thus parallel groups to *H. vulgare* and *H. hexastichum*. The various Goldthorpes represent this group in this country.

H. decipiens has the lateral florets still further reduced. Its varieties show mere traces of these structures with a pair of glumes at the base. No sexual organs are present in these rudimentary spikelets. The median florets are fertile. The group is cultivated in Abyssinia, but it is little known here and never cultivated as a crop. At present the lax-eared varieties are fairly numerous and the wide-eared so rare that no group parallel with *H. zeocriton* has been made to receive them. As will appear later there is no difficulty in raising such forms.

[1] Morton's *Cyclopaedia of Agriculture*, 1869, p. 183.

Besides the characters afforded by the fertility or otherwise of the spikelets and the internode length, there are many others. The paleae provide some of considerable importance. In most barleys the paleae are prolonged to form long, serrated awns, the ear being then known as bearded. In some few varieties the awns are smooth, whilst in others they are wanting, the variety being then beardless. Such forms must not be confused with the so-called beardless barleys which shed their awns on ripening. The paleae may also be "trifurcate" as in the case of Nepaul barley. The term is used to describe a small hood-like swelling either at the apex of the paleae or borne on a short awn. At the base of this hood (kapuzen) are a pair of pointed auricle-like processes, and within the structure itself there is a rudimentary flower which may at times set grain. If the palea may be considered to be a modified leaf sheath, the auricles correspond with those of the leaf and the flower is epiphyllous, being borne on the leaf blade[1].

The colour of the paleae of different varieties of barleys is often very characteristic. In our cultivated forms it is yellow or yellowish grey as in the Archer barley. Black and purple paleae are however common. As a rule this purple colour is most marked before the grain is ripe. The extent to which it persists appears to depend on the weather conditions, but even when it is faded some traces usually remain which enable one to say whether the paleae were purple or not. The black colour also develops as the ears ripen, but it is persistent.

These coloured paleae are generally associated with coloured grain, black or purple eared barleys generally having grain varying in colour through shades of violet, brown-violet or green-violet. It appears that these colours in the paleae are associated with those of the grain. In addition to colour characteristics the grain provides others. The shapes differ to a certain extent, but little attention has been paid to this at present, and the caryopsis may, or may not, be fused with the paleae; in the latter case the grain is described as naked, in the former, for the sake of convenience, I have called it "trapped."

The glumes in the majority of the barleys are small and not conspicuous, but in some few varieties they are ovate lanceolate in shape and occasionally more or less awned. The rachilla provides a useful pair of characters to those who handle barleys commercially. It may be long and almost hairless, or it may be short and covered with silky hairs. The former condition is known as "smooth," the latter

[1] Cf. Raciborski, *Bot. Centrb.* Vol. xc. p. 407 (abstract).

as "bristly." Other characters of less importance will be referred to later.

The operation of crossing barleys is not particularly difficult, but it cannot be carried out as rapidly as in the case of wheat. After various trials the following method was adopted. The sheath was split open when the awns were emerging and the ear freed from it. All except from eight to twelve median florets were stripped from the rachis, and these were then emasculated. The simplest method for removing the stamens is to cut off the apex of the paleae. The proper height to make the cut can as a rule be determined by holding the ear up to the light, when the position of the stamens can be detected. Through the opening so made into the flower, the stamens can be removed without tearing the paleae. Attempts to open the paleae to remove the stamens so often resulted in failure that this method was abandoned towards the end of the experiments. To pollinate the stigmas, stamens just at the stage of breaking were inserted into the opening at the apex of the paleae. These were obtained as in the case of wheat, by making use of the peculiar elongation of the filaments when the stamens are ready to dehisce. The tips of the florets of ears of the variety required as the male parent were cut off and laid aside for a few minutes when, if sufficiently ripe, the stamens protruded and could readily be removed. After pollination the ears were enclosed in small bags of thin paper water-proofed by soaking in paraffin wax. These were generally allowed to remain on the ears until harvest. As in the case of wheat pollination rarely failed in fine weather, but when wet, failure was the general rule.

The arrangement of the plots and the records was the same as that described in the wheat-breeding experiments.

Brief descriptions of the varieties used as parents are appended as these are little known to those who have not given special attention to this group of plants[1].

H. hexastichum.

H. pyramidatum (Kcke.). Ears dense, pyramidal. Paleae awned and white.

H. Schimperianum (Kcke.). Ears dense, black, awned paleae enclosing the grain which is black also, rachilla bristly.

[1] Detailed descriptions will be found in Körnicke and Werner in Bde. I. and II. of their *Handbuch des Getreidebaues*, Berlin, 1885, or in Körnicke, *Die Hauptsachlichsten Formen der Saatgerste*, Bonn, 1895.

H. japonicum. Ears dense, paleae yellow, with broad awns, grain naked white.

H. densifurcatum. Ears dense and six rowed, paleae black and trifurcate, glumes lanceolate.

H. eurylepis (Kcke.). Ears dense, glumes ovate-lanceolate and awned.

H. hexasticofurcatum (K. H.). Paleae trifurcate, white, enclosing the grain.

H. vulgare.

H. vulgare (L.), *H. pallidum* (Ser.). Ears lax, paleae awned, yellow, enclosing the grain which is light in colour.

H. himalayense (Rittig.). Ears lax, paleae grey or yellow, grain naked and violet.

H. trifurcatum (Schl.). Known as Nepaul barley or sometimes as Nepaul wheat. This variety is the "dreigablige gerste" of German writers. Ears less lax than in most varieties of the group *H. vulgare.* Paleae ending in hood-like expansions containing supernumerary florets (trifurcate paleae), grain naked.

H. aethiops (Kcke.). Ears narrow, six rowed, paleae black, trifurcate, grain naked, black.

H. atrum (Kcke.). Ears lax, six rowed, paleae and grain black, trifurcate.

H. violaceum (Kcke.). Ears lax, paleae awned, purple when unripe, changing to dull yellow or grey as a rule when ripe, but in some seasons retaining a purple tinge. Grain naked, purple to greenish violet in colour.

H. intermedium.

H. transiens (Kcke.). Ears dense, lateral florets small but fertile and awnless.

H. Haxtoni[1] (Kcke.). Median florets fertile and awned, lateral florets small but fertile, awnless.

H. distichum.

H. nutans (Schubl.). Chevallier barley. Ears lax, paleae awned, white, enclosing the grain, rachilla smooth.

H. rigens (K. H.). Similar to Chevallier, but with awns which are smooth from below.

[1] Morton's *Cyclopaedia of Agriculture*, 1869, p. 183.

H. inerme (Kcke.). Ears lax, paleae awnless, white. One of the few varieties of barley in which the awn is wanting. It originated from a cross of Rimpau's between *H. Steudelii* and *H. trifurcatum.*

H. nigrosubinerme (Kcke.). Paleae practically awnless, black.

H. ianthinum (Kcke.). Ears lax, paleae blackish, awned, grain naked and violet.

H. utriculatum (K. H.). Ears lax, paleae light in colour, trifurcate, the hoods unusually large and sessile. Grain naked. The supernumerary flowers are occasionally fertile.

H. nigrescens (Kcke.). Ears narrow, two rowed, paleae blackish, awned.

H. spontaneum (C. Koch). Ears two rowed, paleae with harsh thick awns, grain narrow, enclosed by the paleae, rachis brittle. The variety is a native of Persia, Palestine and other parts of Asia, and it is supposed to be one of the wild prototypes of the modern barleys. In this district it is perfectly hardy, and owing to its habit of shattering when ripe, becomes a weed unless it is harvested early. In the seedling stage it is easily recognised by its strongly twisted foliage which lies on the soil weeks after most varieties have become upright. A more detailed description will be found in Körnicke.

H. zeocriton.

H. zeocrithum[1] (L.). Goldthorpe type. Ears dense, paleae awned, white, enclosing the grain, rachilla bristly.

H. decipiens.

H. deficiens (Steudel). The variety is typical of the group *H. decipiens.* Spike lax, narrow and, partly owing to the entire suppression of the lateral florets, peculiarly slender. Paleae awned, bright yellow, enclosing the grain. The paleae open more at the flowering period than most barleys, but the variety appears to be truly autogamous.

H. Steudelii (Kcke.). Very similar to *H. deficiens* in shape, but the paleae and the grains are black.

H. Abyssinicum (Ser.). Ears lax, paleae awned, almost white in colour, enclosing the grain, glumes lanceolate and shortly awned.

H. decorticatum (Kcke.). A variety of *H. decipiens* with black paleae and grain; paleae non-adherent to the grain.

[1] Linnaeus, *Species Plantarum* (1753), p. 85.

F. 1 *Generation.*

H. Schimperianum × *H. nutans.* Ear lax but slightly denser than a normal Chevallier, two rowed, paleae and grain black, rachilla bristly, glumes with slight awns.

H. pyramidatum × *H. deficiens.* Ears lax, resembling those of a Chevallier barley, slightly denser than those of the male parent, glumes with short awns.

H. japonicum × *H. Steudelii.* Ears lax and Chevallier-like, with larger lateral florets than those of *H. Steudelii,* paleae black with ribbon-like awns, grain for the most part trapped, violet-black in colour, glumes with small awns.

H. vulgare × *H. Steudelii.* Ears lax, Chevallier-like, paleae black and awned, grain trapped, black.

H. trifurcatum × *H. nutans.* One plant only was secured and this was attacked by Helminthosporium and failed to set any grain. Ears of the Chevallier type, but a little denser, paleae with awns from three to nine cms. in length bearing hoods for the most part, about half-way up, but in some cases terminally.

H. transiens × *H. deficiens.* Ears laxer than those of *H. transiens* but denser than those of *H. deficiens,* lateral florets not as large as those of *H. transiens,* and containing stamens only, glumes with short awns.

H. inerme × *H. Haxtoni.* Ears of the Chevallier type, awnless.

H. nutans × *H. himalayense.* Ears lax, paleae greyish yellow, grain naked and violet. Many of the lateral florets on each of the plants were fertile. Grain not truly naked; on rubbing out an ear some grains escaped from the paleae, but the majority remained enclosed, violet, rachis brittle when ripe.

H. nutans × *H. Steudelii.* Ears lax, lateral florets minute but better developed than those of the male parent, paleae of the median florets black, of the minute laterals white. Grain black.

H. ianthinum × *H. utriculatum.* Ears of the Chevallier form; paleae tinged with violet, trifurcate, the supernumerary florets exceptionally large and occasionally fertile, sessile, grain naked and purple.

H. nigrescens × *H. aethiops.* Ears of the Chevallier type, but with somewhat larger lateral florets which occasionally set grain, paleae black with supernumerary florets on short stalks up to two cms. in length.

H. rigens × *H. atrum.* Ears of the Chevallier type, but in two plants out of a total of eight some of the ears had fertile lateral florets here and there. Paleae black with hoods on stalks some two cms. in length. Grain when divested of the adherent paleae purple.

H. spontaneum × *H. eurylepis.* Ears two rowed, glumes narrow, awned, grain narrow, rachis brittle. At first sight the hybrid seemed identical with *H. spontaneum*, but closer inspection showed the greater development of the awns.

H. spontaneum × *H. hexasticofurcatum.* Ears lax and two rowed. Paleae ending in an awn about two cms. in length bearing a terminal hood and secondary floret, lateral florets larger than those of *H. spontaneum* but not fertile, grain trapped and slender, rachis brittle. The spikes scattered as soon as ripe, but owing to the lack of awns the grains were unable to bury themselves so effectively as those of *H. spontaneum.*

H. zeocriton × *H. nutans.* Ear lax, but less so than a normal Chevallier, nodding, rachilla bristly.

H. nigrosubinerme × *H. hexasticofurcatum.* Ears lax and of the Chevallier type, but with a fertile lateral floret here and there. Paleae black and awnless.

H. deficiens × *H. japonicum.* Ears of the Chevallier type, less dense than those of the male parent, but denser than those of *H. deficiens.* Paleae with broad awns, more or less free from the grain. Grain more elongated than that of *H. japonicum.*

H. deficiens × *H. pyramidatum.* Ears Chevallier-like.

H. deficiens × *H. nutans.* Ears lax, lateral florets minute, but larger than those of the male parent, sexless.

H. deficiens × *H. violaceum.* Ears lax, resembling those of a Chevallier barley, purple when ripening, brown when ripe. Grain trapped, when stripped of the paleae deep violet in colour.

H. deficiens × *H. parallelum.* Ears similar to those of a Chevallier barley, but the laterals a little smaller, glumes with short awns.

H. Abyssinicum × *H. Steudelii.* A typical *decipiens* with black paleae and grain with narrow glumes.

H. Abyssinicum × *H. trifurcatum.* Ears lax, two rowed with the lateral florets similar to those of a Chevallier barley. Paleae white, ending in awns from one to four inches in length which bore either terminally or half-way along their length supernumerary florets. These varied considerably in their development, some contained perfect stamens and ovaries, whilst others were rudimentary with mere traces

of sexual organs. In extreme cases the florets were missing, the awn then being sharply bent about half-way up in the position in which they would, presumably, have occurred. Grain enclosed by the paleae, glumes awned slightly.

H. decorticatum × *H. densifurcatum.* Ears lax and Chevallier-like; paleae black, bearing nearly sessile hoods, lateral florets lighter in colour than the median, grain slender, partially naked and black.

Sexless and Staminate Lateral Florets.

A few crosses have been made between a Chevallier barley (*H. nutans*) and two of the varieties of the sub-species *H. decipiens*, in which the lateral florets are so reduced that it is difficult to distinguish even the paleae as such. These lateral florets are entirely destitute of sexual organs. The crosses were as follows:—

H. nutans × *Steudelii.*
H. Steudelii × *nutans.*
H. nutans × *deficiens.*

The hybrid in all cases bore sexless lateral florets, but the paleae were slightly more developed than in the corresponding parents. In those descended from the black-eared *H. Steudelii* these rudimentary lateral florets showed up like white stitches on a black ground. The F. 2 generation consisted of plants of three types bearing staminate, small or sexless lateral florets. The groups were perfectly distinct from one another, and could readily be sorted. The proportions for each plot were as follows:—

Staminate	Small	Sexless
41	62	20
39	78	36
54	119	56

Taken as a whole these results indicate that the types occur in the ratio of 1 : 2 : 1, though the first quoted is a rather wide departure from the normal. This is in all probability fortuitous, as the reciprocal cross obviously gives the 1 : 2 : 1 ratio. A number of the heterozygotes with the small lateral florets were dissected under a lens, but no stamens, fertile or otherwise, could be detected in any case. Thus the sexless condition might be described as dominant over the staminate if no attention were paid to the slight increase in development of the lateral florets of the heterozygote.

A few sowings of each of these three types have been made. Grains from plants with small lateral florets produced the three types

again in each of the 20 cases tested. A similar number of plants with staminate lateral florets proved to be homozygous, and 15 plants with the rudimentary lateral florets of the parents also bred true to this feature.

Hermaphrodite and Sexless Lateral Florets.

This pair of characters has been investigated in the following crosses:—

(*a*) *H. deficiens* × *violaceum.*
(*b*) *H. japonicum* × *Steudelii.*
(*c*) *H. vulgare* × *Steudelii.*
(*d*) *H. Abyssinicum* × *trifurcatum.*
(*e*) *H. pyramidatum* × *deficiens* and its reciprocal.
(*f*) *H. deficiens* × *parallelum.*
(*g*) *H. decorticatum* × *densifurcatum.*

The F. 1 plants bore small lateral florets which, when examined in 1903, (*a*—*d*) were considered to be sexless, as the stamens appeared to be rudimentary and incapable of producing normal pollen grains. This view was negatived in the following season when a further series of F. 1's was available for more detailed examination, as well as a series of F. 2's. These showed conclusively that the normal form of the heterozygote was characterised by the formation of truly staminate laterals. From the systematist's point of view it would be described as *H. distichum* or *zeocriton* according to the laxness or denseness of the ears.

In the F. 2 generation the fully fertile, the staminate, and the rudimentary lateral bearing types were met with in the proportions of 1 : 2 : 1. The actual figures given in the same order as the list above were:—

26 : 52 : 23, 16 : 47 : 21, 30 : 65 : 25, 39 : 85 : 44,
54 : 114 : 55, 80 : 147 : 74, 47 : 98 : 44.

The three groups were perfectly distinct from one another, and no forms occurred which could be described as doubtfully belonging to one group or another. A subsequent generation was raised from each type. In all, 48 cultures of forms with hermaphrodite lateral florets and 62 with rudimentary lateral florets were grown, and in every case, no matter what the parents, they bred true to these characters: 110 cultures of the heterozygote with staminate laterals were also raised, and these, without exception, produced the three types once more. Complete

statistics of these groups were not considered necessary, as from an inspection of the plots it was evident that the types again occurred in the ratio of 1 : 2 : 1. In one case, *H. pyramidatum* × *deficiens*, an F. 4 generation containing several hundred plants was grown, all of which proved true to the original six row type sown. In addition to the series already described, a cross was made between *H. transiens* with small, but hermaphrodite, lateral florets and *H. deficiens*. The F. 1 again produced staminate lateral florets. Only one poor plant was raised, and this gave in the next generation seven plants with small, but fertile lateral florets, 13 with staminate, and 15 with rudimentary lateral florets. The numbers are too small to lay much stress upon, and in all probability the somewhat large departure from the general ratio is due to the smallness of the count. All of the forms with staminate laterals were sown, and they in turn split off the forms with hermaphrodite and rudimentary lateral florets.

Staminate × *Hermaphrodite Lateral Florets.*

The varieties with staminate lateral florets include the Chevallier and Goldthorpe types, that is the so-called two rowed barleys, whilst those with hermaphrodite lateral florets include the six row and the so-called four rowed barleys. As these are the types in general cultivation, there are naturally a considerable number of crosses between them recorded in the earlier literature of the subject, nevertheless it is difficult to determine the behaviour of these characteristics from the descriptions given. Rimpau's[1] account of a cross between Pfauengerste and Gabelgerste (Fan or Peacock and Nepaul barley) is perhaps one of the most satisfactory of these. He describes and figures the F. 1 as being intermediate between a two row and a four row barley, the lateral florets being partly fertile. These florets, further, were hoodless, whilst those occupying the middle position of the group of three were hooded. In a natural cross between *H. pallidum* and *H. nutans* he again observed this same form in the heterozygote. Tschermak[2] found that the two row condition was dominant over the four and six row in the F. 1, but in the generation raised from it the splitting was impure. Wilson[3] in the case of Standwell and Bere found evidence that "seemed to warrant the assumption that the two row character was recessive," but in the succeeding generation the form of the individuals appears to have been

[1] Rimpau, *Landw. Jahrb.* Bd. xx. p. 356.
[2] Tschermak, *Deutsche Landw. Presse*, xxx. Nr. 82.
[3] Wilson, *Journ. Agric. Sci.* Vol. II. p. 86.

more or less indeterminate. In a second example where Standwell was crossed with a six row barley, the six row character was recessive.

In a former paper dealing with this subject I have described a series of F. 1 plants and one F. 2 generation, which indicated that the two row type was dominant over the six row. As will be shown later this view is not altogether correct.

These characters have been investigated in the following series of crosses:—

H. Schimperianum × *nutans.*
H. nigrosubinerme × *hexasticofurcatum* and its reciprocal.
H. spontaneum × *hexasticofurcatum.*
H. trifurcatum × *nutans.*
H. spontaneum × *eurylepis.*
H. rigens × *atrum.*
H. nutans × *himalayense.*
H. nigrescens × *aethiops.*
H. persicum × *elongatum.*

The F. 1 generation of the first five examples was grown in 1904, and all of the plants appeared to possess the ordinary staminate lateral florets of the two rowed parent, for no grain was set in any case. The six rowed type was thus recessive to the two rowed, and this view was borne out by the fact that an F. 2 generation of the cross between *H. hexastichum* × *nutans* consisted of two row and six row forms in the ratio of 3 : 1. In the remaining four examples the F. 1 grown in the following year was, from the morphological point of view, a form of *H. intermedium,* inasmuch as the lateral florets were fertile, smaller than the median florets and destitute of awns or hoods. Among the F. 2's of the previous series were many examples with similarly developed ears.

The F. 2 of *H. Schimperianum* × *nutans* was chosen for a detailed examination of the degree of fertility of the lateral florets. It was found to contain 49 individuals with the large fertile lateral florets, and 46 with the small staminate lateral florets characteristic of the two parents. Of the remaining 99 individuals, 16 belonged to the group *H. intermedium,* and 83 bore either a few fertile, and small, lateral florets or florets which were larger and more pointed than those of the parent with the staminate lateral florets. Sowings were made of each of the *H. intermedium* types, and also of 20 individuals showing varying degrees of development of the lateral florets. In some of these a few lateral florets were fertile, but the majority were grainless. The whole series

proved to be heterozygotes, each sowing giving the three expected types. The totals for the whole series were 103 with fully fertile, 211 with small but more or less fertile, and 98 with staminate lateral florets similar to those of the parent *H. nutans.* No differences were detected in the progeny from ears in which all the small lateral florets were fertile, and those in which no grain was set. From this there can be no doubt that the F. 2 generation consisted of six row, intermediate and two row forms in the proportion of 49 : 99 : 46, or in the ratio of 1 : 2 : 1.

In the subsequent examination of such forms those individuals with enlarged lateral florets have always been considered as heterozygotes. Thus in *H. nutans* × *himalayense* the F. 2 consisted of 40 plants with large fertile laterals, 80 with smaller and intermediate, and 41 with staminate lateral florets. Similar indications of this 1 : 2 : 1 ratio have been observed in the other crosses, but they still have to be examined statistically[1].

A considerable number of the typical six row and two row forms from the first five crosses have been tested in the F. 3. In all the trials amount to 85, and in each case the individuals tested have proved to be homozygous.

The heterozygote therefore of forms with hermaphrodite and staminate lateral florets is potentially hermaphrodite, and of the type known to systematists as *H. intermedium.* As a general rule the small lateral florets are hoodless and awnless, though in some cases traces of these structures have been observed, more particularly in a cross between *H. rigens* and *atrum.*

Investigations on the anomalous results of the F. 1 generation grown in 1904, are still being carried out. As far as the tests have gone at present, the incomplete development of the lateral florets appears to have been due to external conditions. Owing to a shortage of space the grains were planted rather closely together and somewhat late in the season. In the following year when the hybrids were typical *H. intermedium* forms, the grains were planted at six inch intervals in rows a foot apart, and, as many failed to germinate, the plants had every opportunity for vigorous development. That the space at the disposal of the plant largely determines the degree of development of the lateral florets of the heterozygote is also indicated by the fact that when F. 2's are standing thinly in the plots, there is not such a large number

[1] The most aberrant result met with so far is in the case of *H. nigrescens* × *aethiops* where the proportions are 24 : 65 : 26.

of individuals with poorly developed lateral florets as in the case described in detail above.

One cross has been made to determine the results of crossing *H. intermedium* with a variety with staminate lateral florets. The parents in this case were *H. transiens* and *H. inerme*. The F. 1 bore staminate lateral florets. The F. 2 consisted of individuals with lateral florets similar to those of the parents and in addition of true six row barleys. The result was found to be due to the fact that the *H. inerme* used as a parent was in itself a heterozygote, for it was throwing six rowed barleys as well as the type. The number of individuals raised in the F. 2 was too small to attempt to unravel this complication, and the experiment was temporarily abandoned.

"*Hooded*" *or Trifurcate and Awned Paleae.*

Examples of parents carrying one or the other of these characters are provided by the following crosses:—

(*a*) *H. Abyssinicum* × *trifurcatum*.
(*b*) *H. spontaneum* × *hexasticofurcatum*.
(*c*) *H. trifurcatum* × *nutans*.
(*d*) *H. decorticatum* × *densifurcatum*.
(*e*) *H. ianthinum* × *utriculatum*.
(*f*) *H. rigens* × *atrum*.
(*g*) *H. nigrescens* × *aethiops*.

The F. 1 was in all cases hooded, the supernumerary florets being almost sessile in *b*, *d*, *e*, *f* and *g*, whilst in *a* and *c* they were borne on awns from half to four inches in length. The length of the awn varied considerably on one and the same ear.

The development of the supernumerary florets when borne on the awns was not so complete as in the parents, and frequently one was missing here and there in an ear, its position being then marked by a kink in the awn.

In the F. 2 generation hooded and awned forms were produced in approximately the ratio of 3 : 1. Thus the series taken in order gave the following figures:—113 : 43, 57 : 30, the F. 1 plant of *c* died before maturing any grain, 143 : 46, 91 : 29, 201 : 65, 96 : 37, or a total of 701 : 250 equivalent to the ratio of 2·8 : 1.

In the F. 2 generation of *a* and *c* there was considerable variation in the length of the awns carrying the supernumerary flowers, but no indications could be detected of segregation into individuals with

sessile hoods, awned hoods, and true awns only. In the case of the cross between *H. Abyssinicum* and *H. trifurcatum*, no plants with hoods as sessile as those of the parent were found in either the F. 2 or F. 3 generation. Further no plants with hermaphrodite lateral florets bore awns except on the median florets. Such individuals from a systematic point of view would be classed in the group *H. intermedium.* In the parent the fertile lateral florets are smaller than is generally the case with forms of *H. vulgare*, and the hoods of these florets are rudimentary or wanting, so that it is possible that the proper position of *H. trifurcatum* is in the group *H. intermedium*, of which at present it would form the only hooded representative. Unfortunately the only other cross with this variety as a parent died in the first generation, so that no other data are for the time being obtainable. Where the hoods of the lateral florets are well developed as in *H. hexasticofurcatum, atrum* or *aethiops*, the corresponding fully awned florets were met with in all cases.

In the F. 3 generation the 45 awned types of various descent tested were found to breed true to this character, whilst of the same number of hooded types, 16 were pure, and 29 gave a mixture of hooded and awned individuals. Six individuals with hoods borne on awns of two or more inches in length from *H. Abyssinicum* × *trifurcatum* which were tested, all bred true to this character, though in three of the cases they split into six row and two row types. The permanence of this feature suggestive of a blend of the parent characteristics was not expected, and no explanations can yet be offered to account for it.

Black and White Colour in the Paleae.

The fact that the black colour is dominant over white is evident from many pre-Mendelian crosses. Cases are described for instance, by Rimpau[1], in which the hybrid was black, and black and white forms appeared in the progeny. More recently Tschermak[2] has investigated the inheritance of these characters, and shown that the black and white forms occur in the ratio of 3 : 1 in the F. 2. The following crosses are between black and white varieties:—

H. japonicum × *Steudelii.*
H. vulgare × *Steudelii.*
H. Abyssinicum × *Steudelii.*

[1] Rimpau, *ibid.*
[2] Tschermak, *ibid.*

H. Schimperianum × *nutans.*
H. nigrosubinerme × *hexasticofurcatum.*
H. nutans × *Steudelii.*
H. rigens × *atrum.*
H. persicum × *elongatum.*

In each case the heterozygote was as black as the black parent, and in the F. 2 the expected black and white forms appeared in the ratio of 3 : 1. The figures obtained were as follows:—68 : 24, 95 : 25, 32 : 8, 154 : 55, 29 : 8, 94 : 29. Statistics for the last two crosses have not been obtained. Some 89 cultures of extracted whites have been grown without a single black-eared individual being produced, and in one case such a white form has been used for further crossing with white without producing any tinted individuals. The black forms proved to be pure with regard to this character or else heterozygotes. Twenty-four of the former class were obtained in a trial sowing of 75 extracted blacks.

Where the variety *H. Steudelii* was used as a black parent, it is worth noticing that the sexless lateral florets, produced when the second parent was *H. nutans*, or the staminate laterals when *H. vulgare* was the parent, were in all cases white. This same feature appeared in the heterozygotes of the two following generations. Up to the present white lateral florets on otherwise black ears have not been obtained in these experiments as stable forms, but they exist as such in such varieties as *H. pictum.*

Purple and White Paleae.

In several varieties of barley the paleae, before the grain begins to ripen, is a vivid purple colour. As ripening progresses this colour disappears, and by the time the crop is ready for harvesting the paleae are a dingy grey colour, showing, however, some signs of its original purple colour. It is advisable therefore to determine this feature in the crop at an early stage.

The crosses made to determine the inheritance of this purple colour were between *H. deficiens* and *H. violaceum* and *H. ianthinum* and *H. utriculatum.*

In each case the F. 1 was as purple as the purple parent, and its descendants consisted of individuals with purple or white paleae. In the first case the ratio of purple to white was 70 : 28, in the second, 91 : 29.

An F. 3 generation of the first cross only has been raised. Twelve white individuals produced only white offspring, whilst three out of 12 purples were pure, and the remaining nine threw purples and whites. No indications of intermediate shades between these two were met with in any case, so that purple may be described as simply dominant over white.

Colour of Grain.

Black colouring in the paleae appears to be associated with colour in the grain. Up to the present no cases have been met with amongst the hybrids in which light grain occurs on plants with dark paleae or dark grain on plants with light paleae[1]. In wheats, on the contrary, colour (red) in the grain is independent of the coloured or colourless condition of the glumes.

Under these circumstances coloured grain is dominant over colourless. It is doubtful whether this provides a complete expression for the facts observed, inasmuch as the colouring is very variable in the F. 2 generation. Thus ears with black paleae may contain grain in which the colour of the caryopsis is black, purple, or violet-green. The latter shades may be very faint, and then they are difficult to distinguish with certainty from the colourless grains. If, however, all grains showing any colour are classed as dark, those without as light, fair approximations to the 3 : 1 ratio are obtained. Further, plants producing colourless grains have invariably bred true to this character.

Narrow and Broad Glumes.

In the majority of the barley varieties the glumes are narrow, but some few have ovate-lanceolate glumes. These characteristics have been investigated in :—

H. Abyssinicum × *Steudelii* and *H. Abyssinicum* × *trifurcatum*.

The glumes of the F. 1 were in both cases narrow, and in the F. 2 narrow and broad glumes appeared in the approximate ratio of 3 : 1. In the first case the figures obtained were 29 : 11, in the second, 127 : 45. A few F. 3 cultures were raised from the latter cross with the result that all the broad glumed forms bred true to this character, whilst three out of eight of the narrow glumed forms proved to be pure.

[1] In *H. himalayense* coloured grain is found in conjunction with white or greyish-white paleae.

Lax and Dense Ears.

As far as one can determine from the existing records of barley hybrids, lax ears are dominant over dense. Judging from the general appearance of the hybrids raised in the course of these experiments, this would also be the case. But as in wheats this does not give a complete account of the phenomena observed in the F. 2 generation. Where extremely lax and dense ears are crossed in wheat then, as Spillmann was the first to demonstrate, the heterozygote is intermediate in this respect. Where the difference is less pronounced this cannot be traced with any certainty: in fact, cases have been recorded where the F. 1 is laxer than the lax parent, and the F. 2 has contained similar excessively lax forms. In such cases it may be that that laxness and denseness are dependent on more than one pair of characters.

Varying degrees of laxness and denseness of the ears are shown in most of the hybrids already described, but the most suitable cases for examination are provided in the following :—

H. zeocriton × *nutans*.

H. pyramidatum × *deficiens* and its reciprocal.

H. parallelum × *deficiens*.

In each case the F. 1 appeared to be as lax as the lax parent, though subsequent measurements have shown that it is slightly denser. The figures are derived from too small a series of plants to lay any stress on. This is due to the fact that a cross has to be made each time to secure a single plant, and the flowering period is so short that it is a matter of some difficulty to make all the necessary crosses. The F. 2 generation has consisted in each case of plants with ears as lax or as dense as those of the parents, together with a series lying between these extremes, which cannot be satisfactorily classified. Trial sowings of the extremes show that they are homozygous, but the heterozygotes cannot be distinguished with any degree of certainty by inspection only. In order to unravel the story, measurements of a series of F. 2 plants have been made, and the average internode length determined by dividing the length of the rachis by the number of the spikelets borne on it.

The figures for *zeocriton* × *nutans* are :—

mms.	1·6	1·8	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·4	3·6	3·8	4·0	4·2
individuals	0	2	15	24	17	7	26	44	39	23	8	2	2	0

for *H. pyramidatum* × *deficiens* :—

	3	7	14	19	12	42	41	28	15	6	4	2	2	1

H. Schimperianum × *nutans* :—

	0	0	2	7	13	30	24	19	14	18	4	5	0	2

The six row and the two row plants show the same distribution of lax and dense ears, the curves, if plotted separately, being the same in both. The first example quoted is perhaps the simplest. In this there are two groups of plants centred about an internode length of 2·2 and 3·0 mms. respectively. Unfortunately there appears to be no method of analysing such a curve without sowing all the individuals represented in it.

This test has been partially made. All the individuals with internode lengths ranging from 1·8 to 2·6 mms. were planted separately. Two with an average internode length of 1·8 bred true, and 15 of 2·0 mms. also bred true; of 24 of 2·2 mms. 20 were homozygous; of 17 of 2·4 mms. 15 were homozygous; of 7 of 2·6 mms. 4 proved heterozygotes. The pure dense forms thus fall into a series with internode lengths as follows:—

mms.	1·6	1·8	2·0	2·2	2·4	2·6
No. of plants	0	2	15	20	15	3

giving, considering the numbers in question, a singularly uniform Quetelet curve. The total number of dense-eared individuals was thus 55 in a generation consisting of 209, or a sufficiently close approximation to the 1 : 3 ratio.

No analysis of the group centering around 3·0 mms. has yet been attempted. Judging from the uniformity of the curve it would appear to consist of a single group, showing the dominant lax character only, but no definite statement as to this can be made at present. In other cases still to be described and investigated more fully, the measurements seem to indicate the existence of three groups of individuals with lax, intermediate and dense ears. The limits of each of these groups overlap, and the curves plotted from measurements of the F. 2 generation show three distinct summits.

It seems probable that if this method of analysing the F. 2 generation were capable of general application, many of the cases where it appears to be indeterminate could be explained.

Adherent and Non-adherent Paleae.

In the following cases one of the parents is naked-grained, that is, the caryopsis is set free from the paleae on thrashing, as it is in the majority of the wheats:—

H. deficiens × *violaceum.*
H. Abyssinicum × *trifurcatum.*

H. nutans × *himalayense.*
H. nigrescens × *aethiops.*

The heterozygote appears to be more or less intermediate in this respect between its parents[1]. The grains are separable from the paleae with some difficulty, and they do not rub out cleanly, as for instance, in such a typical naked barley as *H. trifurcatum.* If a handful of grain is rubbed, some grains separate completely, in some the paleae are rubbed from the back of the grain only, and others remain completely enclosed. The extent of the separation of the paleae depends to a considerable degree on the amount of weathering the ears have been exposed to.

The generation raised from the hybrids is again difficult to classify. This was also noticed by Rimpau.

A detailed examination of this pair of characters was made with *H. deficiens* and *H. violaceum.* Counting all individuals in which the paleae could be separated perfectly, or partially, by rubbing as naked-grained, 76 were placed in this group, and 25 in the group with trapped grains. Six and two rowed, coloured and colourless forms of each occurred. Sowings were made from 20 of the plants with naked grain, and all of these reproduced that feature. A similar number of trials were made with plants in which the grain was trapped, and with ten plants with partially enclosed grain, it being assumed that these latter would prove heterozygotes and the former homozygotes. It was found, however, impracticable to draw a line of demarcation between these groups, for some cultures in each were heterozygotes and some homozygotes.

An examination of the F. 2 generation of *H. nigrescens* × *H. aethiops* was made in a different manner. Instead of subjecting the grain to violent rubbing, it was stripped from the rachis and then pressed from the base upwards. Under this treatment the grain of the naked parent *H. aethiops* was readily set free from the paleae. Of 72 individuals so tested 21 were found to have truly naked grain, whilst in 51 the grain was more or less trapped. Taking the evidence as a whole, it appears that the trapped condition comes very close to being dominant over the naked, but in view of the difficulty of separating the forms in the F. 2 it is best treated, for the present, as giving an intermediate with partially naked grain.

[1] Cf. Liebscher, *ibid.*

Brittle and Tough Rachis.

In many varieties of barley the rachis is more or less brittle, but this character is only strongly marked in *H. spontaneum.* In this variety the rachis is so fragile that each group of spikelets falls as a whole as soon as ripe. The apical groups are the first to break off. The narrow grains with the stiff awn still attached fall point downwards to the ground and quickly bury themselves. This variety has been crossed with *H. hexasticofurcatum* and *H. eurylepis.* In the first case the F. 1 was a two rowed barley with trifurcate paleae, in the second, at first sight it appeared to be identical with *H. spontaneum.* As the ears ripened the rachis shattered, and the spikelets were set free as in the case of the parent. Those with trifurcate paleae were unable to bury themselves.

The F. 2 generation consisted of numerous types, as the parents differed in several features, and the brittle or tough character was distributed impartially over these. Thus brittle or tough forms with six or two rows, with hoods or awns, or with lax or dense ears were produced. The proportions were estimated at an early stage of ripeness as it was desirable to prevent the shedding of grain as far as possible, for previous experience had shown that these brittle-eared forms might readily become weeds under our conditions of cultivation. As a result of the earliness of the count some ears were not sufficiently forward enough to be classed with any certainty, and these have been neglected. In the first cross the proportions were 56 brittle to 16 with a tough rachis. In the second case the corresponding figures were 22 and 6. No further generations of these crosses have been cultivated.

In connexion with this pair of characters it should be mentioned that several of the F. 1 generations have been more brittle in the rachis than either of the parents. This was particularly the case in a cross between *H. nutans* and *H. himalayense*, the plants resulting from this mating being almost as brittle in the rachis as *H. spontaneum.* Its descendants were in a few cases slightly brittle, but it has proved impossible to sort them satisfactorily.

Awnless × Hooded Paleae.

The inheritance of the awnless character has not been investigated in sufficient detail at present, and further experiments are in progress with the object of removing this defect. The sole cross between

varieties showing these characters is that between *H. nigrosubinerme* and *H. hexasticofurcatum.* The awnless character is one of some interest, inasmuch as the character is a particularly recent one, having first come into existence in a series of hybrids raised by Rimpau from a barley with trifurcate paleae. The F. 1 generation of the cross was as awnless as the parent, and resembled it in other details to such an extent that it appeared to be possible that the cross had missed. One poor plant only was obtained which set little grain. The plants produced from this were as follows:—27 beardless, 7 hooded, and 7 awned. The beardless plants in several cases showed very small barb-like outgrowths at the ends of the paleae or on short awns of about one cm. in length. These were, as a rule, so small that a lens was necessary to distinguish them with certainty. The occurrence of the true awns was unexpected, and at first sight appears to be due to the parent itself being a heterozygote. This plant has unfortunately not been kept under observation, so in order to clear the matter up further crosses have been made.

Other minor characters.

In experiments on heredity it is inevitable that characters besides those being actually investigated should come under notice. In the case of the barleys these lesser features appear to be especially numerous, but for the most part time has been wanting to follow the inheritance of these in any detail, and attention has been directed in the main to characters of immediate importance.

Still the following short account of their inheritance will probably prove correct. Long × short grains gives long-grained forms in the F. 1 and an excess of these forms in the F. 2. This has been observed in the crosses with the long-grained *H. spontaneum.*

The broad ribbon-like awns found in such varieties as *H. japonicum, H. himalayense* and *parallelum* are dominant over the narrow forms occurring in the majority of the barleys. No attention has been paid to these characters in the generations raised from the hybrids. Similarly the slight awns seen on the glumes of many varieties are possibly dominant over the lack of awns found in others.

The bristly type of rachilla found in such varieties as the Goldthorpes is dominant over the smooth rachilla occurring in the Chevallier varieties. No figures have been obtained from the F. 2 generation but from data observed in the F. 3 the bristly rachilla is in excess of the smooth.

There is no evidence to show that the Goldthorpe type of ear is necessarily associated with the bristly rachilla.

In addition to these characters the percentage of nitrogen present in the grains of F. 1 and F. 2 individuals raised from parents showing marked difference in this respect, has been followed out in some detail, as on this it is practically certain the malting value of barleys depends to a great extent. A preliminary discussion of this has already been published, and it is hoped that full details will soon be available[1].

[1] Biffen, *Journ. Brew. Inst.* Vol. XII. 1906, p. 344.

LOSSES IN MAKING AND STORING FARMYARD MANURE.

By T. B. WOOD, M.A.,

Reader in Agricultural Chemistry in the University of Cambridge.

The objects of the investigation described in this and the following pages are two: to determine the losses in constituents of manurial value which take place in the process of making and storing farmyard manure in the ordinary course of good farming practice, and to determine the proportion of the constituents of manurial value of such purchased foods as "cakes" which actually find their way on to the land.

With these objects in view, four heifers were fed for a period of 84 days on carefully weighed and analysed diets, and at the end of the experiment the dung was weighed and analysed. From the figures so obtained, the amounts of nitrogen, phosphoric acid, and potash in the foods eaten, can be calculated, and compared with the amounts recovered in the dung.

The actual details of the experiment are as follows:

The experimental feeding began on 31 January 1906, and ended on 25 April 1906, a period of 84 days. During this time two of the animals consumed 13720 lbs. of mangels, 1176 lbs. of hay, and used up 1963 lbs. of straw as food and litter. The other two animals in the adjoining box consumed almost exactly the same amounts of mangels, hay, and straw, and in addition 672 lbs. of decorticated cotton cake.

Samples of all the foods and litter were set apart from time to time during the experiment. At the end of the time these were chaffed, pulped, or ground, as seemed best to meet the case, and small samples drawn for analysis.

The live-weight of the animals was ascertained at the beginning and end of the experiment, and from the live-weight increase, the weights of nitrogen, phosphoric acid, and potash retained in the bodies of the animals were calculated, on the assumption that these amounts would

be the same proportion of the increase in both cases, namely that given by Lawes and Gilbert for young growing animals[1]. This assumption is probably not quite accurate, since the increase in the case of the better fed animals might be expected to contain a greater proportion of fat, and consequently a smaller proportion of manurial constituents.

On 22 May 1906 the dung was sampled. At this time it was in a solid well trodden down condition, just as the animals had left it. The sampling was carried out without disturbing the dung, by cutting out a number of blocks with a hay-knife. These were well mixed, a small sample taken for analysis, and the rest replaced and trodden down. Duplicate samples were taken in this way from each box. The second sampling took place on 6 November 1906, about six months after the first. This time the samples were taken by throwing occasional forkfuls into a barrow, when the dung was being carted out of the boxes on to the land. On this occasion it was also weighed.

The weight of the dung on 22 May was calculated from the analyses, on the assumption that no loss of phosphoric acid had taken place between the two dates of sampling[2]. It was considered that this procedure would introduce fewer errors than the disturbance of the dung which would have been entailed in weighing it on the first occasion of sampling.

The analysis of the dung was carried out as follows:—2500 grams were dried by spreading out on a large enamelled iron tray which was kept on a hot plate at about 60° C. in a good draught. The drying was completed in the steam-oven. The dried dung was chaffed, and finally ground in a mill. Nitrogen was estimated in the dry-matter by Kjeldahl's method, phosphoric acid and potash in the ash of the dry matter, the latter by Laurence Smith's method. Nitrogen in the form of ammonia was estimated in the fresh dung by shaking 500 grams with 1000 c.cm. of approximately decinormal hydrochloric acid. The liquid was strained off through cloth, its total volume calculated by adding to 1000 c.cm. the volume of water contained in 500 grams of dung, as found in the dry-matter estimation, and an aliquot part distilled with magnesia into standard acid.

The boxes in which the animals were housed during the experiment were bricked up to the highest level reached by the dung. Their floors were not cemented, but were made of clay which was well rammed, and through which there could be little leakage of soluble constituents.

The following table gives the figures:

[1] *J. R. A. S. E.*, 3, VIII. 702. [2] Cp. Dyer, *J. Agr. Sc.* I. i. 111.

	NO CAKE									CAKE FED								
	Weight, lbs.	Percentage composition				Containing by weight, lbs.				Weight, lbs.	Percentage composition				Containing by weight, lbs.			
		Dry matter	Nitrogen	Phosphoric acid	Potash	Dry matter	Nitrogen	Phosphoric acid	Potash		Dry matter	Nitrogen	Phosphoric acid	Potash	Dry matter	Nitrogen	Phosphoric acid	Potash
Mangels—Long Red	13720	13·0	0·128	0·047	0·366	1784	17·6	6·4	50·2	13720	13·0	0·128	0·047	0·366	1784	17·6	6·4	50·2
Hay—Meadow	1176	84·0	1·810	0·410	2·25	988	21·3	4·8	26·5	1176	84·0	1·810	0·410	2·25	988	21·3	4·8	26·5
Straw—mixed	1963	84·0	0·460	0·070	1·810	1649	9·0	1·4	35·5	1863	84·0	0·460	0·070	1·810	1565	8·6	1·3	33·7
Cake—Decorticated Cotton	...	...	...	...	...	...	...	...	...	672	90·0	6·37	3·18	2·40	605	42·8	21·4	16·1
Total in foods and litter	...	...	...	...	...	4421	47·9	12·6	112·2	...	...	...	...	...	4942	90·3	33·9	126·5
*Estimated in l.w. increase	152	75·4	2·54	1·72	0·22	115	3·8	2·6	0·4	326	75·4	2·54	1·72	0·22	246	8·2	5·6	0·8
Excreted by animals	...	...	...	...	...	4306	44·1	10·0	111·8	...	...	...	...	...	4706	82·1	28·3	125·7
Found in fresh dung	11333	22·85	·318	·075	·855	2590	36·0	8·5	96·9	12370	24·1	·574	·190	† —	2969	71·0	23·5	—
Loss	...	...	...	...	...	1716	8·1	1·5	14·9	...	...	...	...	...	1737	11·1	4·8	5·1
Percentage recovered in increase	...	...	...	...	...	2·6	8·0	20·6	0·4	...	...	...	...	...	5·0	9·0	16·4	0·6
,, ,, dung	...	...	...	...	...	58·6	75·2	67·5	86·4	...	...	...	...	...	60·0	78·5	69·3	—
,, loss	...	...	...	...	...	38·8	16·8	11·9	13·2	...	...	...	...	...	35·0	12·5	14·3	—
Found in dung after 6 months	8075	23·2	·383	·105	1·01	1873	30 9	8·5	81·6	8106	25·3	·576	·290	1·11	2051	46·7	23·5	90·0
Loss in storage	...	...	...	...	...	717	5·1	0·0	15·3	...	...	...	...	...	918	24·3	0·0	30·6
Percentage loss during storage	...	...	...	...	...	16·2	10·6	0·0	13·6	...	...	...	...	...	18·6	26·9	0·0	24·2
,, ,, in making and storing	...	...	...	...	...	55·0	27·4	11·9	26·8	...	...	...	...	...	53·6	39·4	14·3	28·3
,, actually applied to soil	...	...	...	...	...	42·4	64·6	67·5	72·8	...	...	...	...	...	41·6	51·6	69·3	71·1

* Figures for growing animals. *J. R. A. S. E.* 1897, 3, VIII. 701.

† A mistake was found in this estimation, and the sample had unfortunately been destroyed before the analysis could be repeated.

The losses which occur in making farmyard manure.

The following figures abstracted from the large table give information on this point:

Dung made by	Amounts not recovered in dung or in increased live-weight per 100 parts consumed in food and litter			
	Dry matter	Nitrogen	Phosphoric acid	Potash
Animals eating roots and hay only...	38·8	16·8	11·9	13·2
Animals eating roots, hay, and cake.	35·0	12·5	14·3	—
Average loss..........................	36·9	14·6	13·1	13·4
Estimated in increased live-weight...	...	8·5	18·5	0·5
Recovered in dung	...	76·9	68·4	86·1

The duplicates agree on the whole very satisfactorily, and the figures shew that it is possible in ordinary good farming practice to recover in the fresh dung about $\frac{3}{4}$ths of the nitrogen, $\frac{2}{3}$rds of the phosphoric acid, and $\frac{7}{8}$ths of the potash contained in the food and litter consumed by the animals. Rather higher proportions would be recovered in the case of older animals, smaller amounts being retained in the live-weight increase.

The state of combination of the nitrogen in poor and rich dung.

Determinations of ammoniacal and non-ammoniacal nitrogen in each of the samples of dung were made as described above. The results are set out below.

Dung made by	Nitrogen per cent. in fresh dung			Percentage of total nitrogen	
	as ammonia	as organic compounds	Total	as ammonia	as organic compounds
Animals eating roots and hay only...	0·028	0·290	0·318	9	91
Animals eating roots, hay, and cake.	0·203	0·371	0·574	35	65

The figures shew very strikingly the effect on the composition of the dung of the use of a concentrated nitrogenous food such as decorticated cotton cake. Its nitrogen is almost entirely digestible, and consequently is excreted in the urine in the form of readily fermentable compounds which rapidly get transformed into ammonia. This leads to a great increase of ammoniacal nitrogen in the dung. Of the 42·8 lbs. of nitrogen contained in the cake consumed by the two heifers, no less than 23 lbs. can be accounted for as increased ammoniacal nitrogen in their dung. Since ammoniacal nitrogen produces a very obvious effect on the crops to which it is applied, this no doubt is the cause of the great reputation of cake-made dung.

The losses which occur during storage.

As already stated the two lots of dung were sampled twice, with an interval of about 6 months between the two dates, including the hottest months of the year. During this period of storage fermentative changes would no doubt be active, though they were minimised by the solid condition of the dung. Analyses were made on each occasion of sampling, and a comparison of the two sets of figures gives information as to the losses produced by fermentations during summer storage, with formation of ammonium carbonate which would get lost by volatilization. These figures are given below.

	Animals eating roots and hay only				Animals eating roots, hay, and cake			
	Dry matter	Nitrogen	Phosphoric acid	Potash	Dry matter	Nitrogen	Phosphoric acid	Potash
Loss per cent. in making dung	38·8	16·8	11·9	13·2	35·0	12·5	14·3	4·1
Loss per cent. in storing dung	16·2	10·6	0·0	13·6	18·6	26·9	0·0	24·2
Total loss in making and storing	55·0	27·4	11·9	26·8	53·6	39·4	14·3	28·3
Retained in live-weight increase	2·6	8·0	20·6	0·4	5·0	9·0	16·4	0·6
Percentage of manurial constituents actually applied to the land ...	42·4	64·6	67·5	72·8	41·4	51·6	69·3	71·1

Several points of considerable interest can be noticed in the above table. Firstly a diet enriched by cake has given a more readily fermentable dung, since the figures shew a distinctly greater loss in

dry matter from the cake dung than from that made without cake. Secondly a still greater loss has taken place in the nitrogen of the richer dung, and this is no doubt connected with the presence in it of greater proportionate amounts of ammoniacal nitrogen as shewn above. The ammoniacal nitrogen was estimated in the rotted dung. The losses of total and ammoniacal nitrogen are given in the following table.

	No Cake lbs. nitrogen	Cake lbs. nitrogen
Ammoniacal nitrogen in fresh dung	3·2	25·1
,, ,, rotted ,, ...	1·9	10·0
Loss of ammoniacal nitrogen during storage..................	1·3	15·1
,, ,, ,, as percentage of total loss...	25	62

The dung of the cake-fed animals contains eight times as much ammoniacal nitrogen as the poorer dung, and about two-thirds of this is lost during storage. This great loss of ammonia accounts for 62 per cent. of the total loss during storage in the richer dung, while in the poorer dung, of the smaller amount of ammonia present only about one-third is lost which accounts for only 25 per cent. of the total loss from this lot of dung. It will be remembered that the assumption was made that no phosphoric acid was lost in storage. There is a certain loss of potash, during storage, and the proportion of the total potash consumed by the animals in food and litter which finally reached the land was only about three-quarters, instead of the whole as estimated by Voelcker and Hall. The amount of phosphoric acid which was found in the rotted dung also falls short of Voelcker and Hall's estimated three-quarters by 6 or 7 per cent. The figures for nitrogen come rather over their limit of 50 per cent. though the amount in the rotted dung from the cake-fed animals cuts it rather fine.

The manurial value of cotton cake.

Since the two pairs of animals consumed practically equal amounts of hay, straw and roots, and since the dung of each pair was similarly treated throughout the experiment, it is possible, taking the no-cake dung as a base-line, to calculate from the figures already given the amounts of the manurial constituents of the cake which were recovered in the dung. The figures for this are given below.

	Dry matter	Nitrogen	Phosph. Acid	Potash
Fresh manure:	lbs.	lbs.	lbs.	lbs.
Animals eating roots, hay, and cake............	2969	71·0	23·5	—
Animals eating roots and hay only	2590	36·0	8·5	96·9
Constituents of cake recovered in dung	379	35·0	15·0	—
Constituents contained in cake eaten	605	42·8	21·4	16·1
Percentage of manurial constituents of cake recovered in fresh dung	63	82	70	—
Rotted manure:				
Animals eating roots, hay, and cake............	2051	46·7	23·5	90·0
Animals eating roots and hay only	1873	30·9	8·5	81·6
Constituents of cake recovered in dung	178	15·8	15·0	8·4
Constituents contained in cake eaten	605	42·8	21·4	16·1
Percentage of manurial constituents of cake which actually reached the land	29	37	70	52

The highly fermentable nature of the cake residue is very evident. While 63 per cent. of the dry-matter of the cake was recovered in the fresh dung, less than half that proportion remained in the dung after 6 months' storage. This proneness to fermentation causes great losses of nitrogen, for the fresh dung contains 82 per cent. of the nitrogen of the cake, as against 37 per cent. in the dung when ready for application to the land. This point seems to be one of considerable practical importance for the following reasons.

When a tenant leaves a farm he is compensated by valuation, not for the total manurial constituents contained in the dung he leaves behind in the yards and buildings and in the soil, but for those constituents only which were presumably derived from the foods, such as cakes, which he had purchased during the last years of his tenancy. In the case under consideration compensation would be paid on the manurial residue of 672 lbs. of decorticated cotton cake. If the compensation due on this were valued on the basis of Voelcker and Hall's estimates, payment would be due on half the nitrogen, three-quarters of the phosphoric acid, and the whole of the potash. But these estimates appear to have been based on experiments in which the measurements made were the proportions of the manurial constituents of the whole diet which were recovered in the dung. In other words compensation is assessed on the assumption that the losses in making and storing dung are practically the same for all the constituents of a diet.

The experiment which is described in the present communication shews clearly that this is not the case, and theoretical considerations seem to support this contention. Thus taking Wolff's figures for the digestibility of the nitrogen of the foods used in the experiment, it appears that of the 48 lbs. of nitrogen consumed as food and litter by the animals receiving no cake, 30 lbs. only would be digested. Subtracting from this the 4 lbs. retained in the form of increased live-weight, it follows that these animals would have excreted in their urine about 26 lbs. of nitrogen. The balance of 18 lbs. would exist in insoluble compounds in the solid excreta, or in the litter. In the same way it is arrived at that the cake-fed animals would excrete about 56 lbs. of soluble nitrogen in their urine and about 26 lbs. in their solid excreta and litter. The richer dung would therefore contain more than twice as much soluble and consequently readily fermentable nitrogen as the poorer, and would be proportionately more liable to suffer loss by fermentation, volatilization, and drainage. If the dung were stored for any length of time before being put on the land, the richer the dung the more rapidly might it be expected to lose nitrogen, and consequently, although more than half the total nitrogen of the food and litter might, with reasonably good management, reach the land, it by no means follows that a quantity of dung, made with the addition of say 1 ton of cake, containing 100 lbs. of nitrogen, would contain 50 lbs. more nitrogen than the dung made from the same amounts of home-grown foods without the addition of the cake. Yet it would be on this 50 lbs. of nitrogen that Voelcker and Hall would estimate for compensation.

In the experiment under discussion, out of 100 lbs. of nitrogen in the cake, only 37 lbs. could be traced in the dung at the time when it was applied to the land. Of this 37 lbs., a considerable proportion was found to be in the form of ammonia, and a still further loss might therefore easily take place if the dung were allowed to lie for any length of time on the surface of the land in dry weather. The actual proportion of the nitrogen of the cake which was recovered in the dung in the experiment varied from 82 per cent. in the fresh dung to 37 per cent. in the rotted dung, and probably even less than 37 per cent. would find its way into the land. The dung was however stored for a longer period, and at a hotter time of year, than is usually the case in farming practice, and the average proportion recovered is therefore probably between the limits found in the experiment for fresh and rotted dung, i.e. between 82 and 37 per cent., and this agrees very well with Voelcker and Hall's estimate of 50 per cent. The point should

not however be lost sight of that the loss in storage and in application to the land falls chiefly on the ammoniacal nitrogen of the cake-made dung, and is so great that the proportion of the nitrogen of the cake which actually finds its way on to the land may, without any flagrant mismanagement, very easily fall below 50 per cent.

Summary.

Two pairs of young heifers were fed on a weighed and analysed diet, and their dung was sampled and analysed both in the fresh and in the rotted states.

It was found that the fresh dung contained about $\frac{3}{4}$ths of the nitrogen, $\frac{2}{3}$rds of the phosphoric acid, and $\frac{7}{8}$ths of the potash consumed by the animals in food and litter.

The dung made by the cake-fed animals was found to be more readily fermentable, and consequently more liable to loss during storage, than that made by the animals fed on roots and hay only.

The loss was found to fall chiefly on the ammoniacal nitrogen in which the cake-made dung is comparatively very rich.

Taking as a base-line the amounts of nitrogen and phosphoric acid in the dung of the animals fed on roots and hay only, it was found that the fresh dung of the cake-fed animals contained 82 per cent. of the nitrogen, and 70 per cent. of the phosphoric acid, of the cake they had consumed.

So great however was the loss of ammoniacal nitrogen from the cake-made dung, that after 6 months' storage under cover in the solid undisturbed state in which it was left in the boxes by the animals, only 37 per cent. of the nitrogen of the cake still remained in the rotted dung.

Dung is not usually kept so long as this, nor through such a hot time of the year, so that the average loss will probably be less than that found in the experiment, and $\frac{1}{2}$ the nitrogen of purchased foods may very well be the average amount recovered in the dung.

The experiment shews however that, without any very flagrant mismanagement, the proportion recovered may fall considerably below $\frac{1}{2}$, especially if the dung suffers further loss while lying on the surface of the land in dry weather.

NOTE ON MENDELIAN HEREDITY IN COTTON.

By W. LAWRENCE BALLS, M.A.,

Botanical Laboratory, Khedivial Agricultural Society, Cairo.

EXPERIMENTS carried out at Ghizeh during 1905 and 1906 shew that the cotton plant follows Mendel's laws of gametic segregation in certain of its characters.

The initial stages of the work were devoted to gathering evidence as to the gametic constitution of the field crop as grown in Egypt. It was found that the individuals of any variety varied extensively except in regard to lint colour. In fact it is doubtful whether any pure types are in cultivation in the country.

An analysis of the offspring of single plants has shown that cross-fertilisation takes place to a certain extent under field conditions and the accumulated effect of this has been to convert the crop into a mass of hybrids. A weed cotton is also present in the crop. This is readily removable by selecting but it would be difficult to eradicate the splitting forms arising from natural crosses with the cultivated varieties.

F. 2 generations of a number of natural crosses have been analysed in respect of their seed characters with results which show that long lint is completely dominant over short. In crosses between distinct types of cottons such as Uplands and Egyptians the lint of the first picked bolls of the F. 1 plants is greater than that of the dominant parent, but in the bolls which ripen later it has the same length as that parent. A similar fluctuation of lint length occurs in cottons grown in a favourable environment, longer lint being found in the bolls of the first picking, whilst in the later various lengths may be found on different seeds even from the same boll. The same cottons grown under less favourable conditions produce lint of a uniform length. Between these extremes the difference may be as much as 12 °/₀.

The inheritance of the colour of the flowers appears to be more complex, and the details of this have still to be investigated. From the evidence obtained at present there is a great probability that time of ripening is a Mendelian character, and if this proves to be the case it should be practicable to check the ravages due to the attacks of the boll-worm (*Earias Insulana*) by raising early maturing varieties.

The breeding of pure types suitable to the needs of the manufacturer and the cultivator will possibly prove a little difficult, owing to the fact that many of the characters of economic importance are dominant.

REVIEWS.

Mendelism. R. C. PUNNETT. [Cambridge: Macmillan and Bowes.] The appearance of a second edition of this admirable little primer is a sign that the biological principles enunciated by the distinguished Abbot of Brünn are engaging an increasing amount of scientific attention. That they are not yet formally applied by the practical breeder may be granted, but that progress has, in the past, been unwittingly effected along Mendel's lines cannot be denied. If Mendel's induction does not furnish the key to all the problems that confront the farmer and gardener, that is simply because the intricacy of the wards of the locks of some of the inner compartments has not yet been fully mastered. But we have now been admitted to the vestibule of knowledge, and it is only a question of time when all the doors will yield.

So far experimentation has been largely confined to animals and plants of relatively small economic importance. Peas and poultry are more easily controlled than cattle and cereals, and so they have received more attention. Biffen's work on wheat is a notable exception, and now we want to see one of the great divisions of farm animals similarly taken in hand. A bigger loaf should have for its complement a corresponding increase in beef and mutton, and that the latter can be secured by the same methods as the former admits of no reasonable doubt. Mr Punnett's book, with its helpful illustrations, may be confidently recommended alike to students and breeders as a clear exposition of the elements of a none too simple subject.

Recent Progress in the Study of Variation, Heredity and Evolution. R. H. LOCK, M.A. [John Murray, 7*s*. 6*d*. net.] Among recent scientific discoveries probably none are of such direct importance to the practical breeder of plants and animals as those which have been made in the field of Variation and Heredity. Pure science is often regarded as dealing with matters which are of no practical importance, but in the work under review a description is given of work which was not undertaken with the direct object of benefiting agriculture, yet

which is of enormous practical value. At the present time the methods used in selection for breeding, and in crossing different breeds, are chiefly empirical, and those engaged in such work do not trouble themselves much with the principles involved. But the recent work here described has shewn that the principles are in themselves simple, and that by understanding and following them the work of the breeder is not only made much easier, but also much more certain of success. No one can read the pages dealing with the results already obtained with maize, wheat and barley, without being convinced that they are of the utmost importance to agriculture.

The book is intended chiefly for the general reader interested in biological science, and all parts of it will not appeal equally to those whose chief interest is agriculture. The first three chapters are devoted to the problems of the Origin of Species, Evolution, and Natural Selection, and give a summary of our knowledge on these subjects before the more recent discoveries were made. The fourth chapter deals with the statistical study of variation, and although it may be difficult reading for those who have no previous acquaintance with the subject, yet it contains conclusions of great importance to the breeder of plants and animals. For example, on pp. 107, 108 the work of Prof. K. Pearson is quoted, shewing that it is impossible to establish a permanent breed simply by selection of minute variations, for if the selection is discontinued the race will tend to degenerate. More important is the conclusion of Prof. Johannsen (pp. 108—112), that within what is considered a single race, there are numerous sub-races each of which can be isolated and cultivated separately, and that the only safe and rapid means of improvement by selection is to isolate the best race by breeding *individual plants* (in this case barley and kidney beans), and choose the plant which gives the best offspring from which to obtain seed.

The following chapters deal with the occurrence of large variations (sports, mutations), and with the manner in which they are inherited. These chapters are the most important in the book, and are full of valuable information. Chapter v. shews how the various races of plants and animals, each of which is distinct from the other races of the same species, have arisen in all cases where we know of their origin by comparatively large definite variations, which have been preserved, propagated and improved by selection till a race is established. Chapter vi. is chiefly historical, shewing the confused state of our knowledge of hybridization before the new work gave the clue.

The central idea of the book is contained in Chapters VII. and VIII., and it is these chapters which are of primary importance to agriculture. They deal with the question of crossing races possessing different characters, and with the way these characters appear in the offspring of such a cross. It is impossible in a short review to give an idea of Mendel's Law which is here described, but an outline may be attempted. We will take an example from Mr Lock's own investigations, described by him on pp. 166—172. In maize the grains may be yellow or white. If a plant belonging to the race with white corn is fertilized by pollen from the yellow-grained variety, all the grains in the cob (the first-crossed generation) are yellow. The yellow colour is thus said to be *dominant.* If now such yellow hybrid grains are sown and the resulting plants are self-fertilized, on their cobs $\frac{3}{4}$ (75 per cent.) of the grains are yellow, and $\frac{1}{4}$ (25 per cent.) are white. When these grains are sown it is found that the white breed true; they are absolutely pure and uninfluenced by their crossed ancestry. Of the yellow grains, $\frac{1}{3}$ (i.e. $\frac{1}{4}$ of all the grains on the cob) are also pure and breed true, and the remainder are dominant hybrids which will yield yellow and white in the same proportions as before. Here then we have a fact not generally appreciated by breeders, that *absolutely pure* individuals may be bred from crossed parents. But another fact of greater importance follows (see p. 174 ff.). If the original parents from which the first cross is made differ in two pairs of characters, e.g. one is yellow and contains starch, the other white containing sugar, then in the first cross all the grains will be yellow with starch, because both these characters are dominant, but when self-fertilized they will yield not only yellow starchy and white sugary, but also white starchy and yellow sugary grains, and a definite proportion of the plants so obtained will breed perfectly true. Here then by crossing we have obtained two new varieties, and by examining the offspring of the *separate plants* we can tell which are pure, and so obtain in two generations a pure race combining the characters of both parent races. The practical importance of this is shewn by the description on pp. 215—221 of the work of Mr Biffen on Wheat, which shews how it is possible to unite such valuable characters as strength with the power of yielding large crops in the English climate, or of combining other important qualities with non-liability to rust. Cases are also given of almost equally interesting discoveries in animals, of which the Andalusian fowl (p. 181) is an example.

A short summary of this kind cannot sufficiently impress the reader with the vast importance to anyone who wishes to undertake the im-

provement of any race of plant or animal of a knowledge of the simple principles involved. The confusion consequent on crossing is shewn to result from the want of discrimination of *separate* characters; when each character is taken alone it obeys simple laws. The fact of the dominance of certain characters is also of great importance, for if a character is completely dominant, the purity of the individual exhibiting it can only be tested by breeding it separately. As an example of the confusion which arises from want of knowledge on this point, we quote the following sentence from a recent paper on the selection of seeds of the cotton-plant. "It is highly important in practice to select more than one excellent plant, as it not infrequently happens that a very fine plant is found having poor transmitting power...." This really means that before selecting a plant for seed one must test its purity in the character required (if that character be 'dominant'), lest the plant turn out to be a hybrid.

The essence of the matter is that single characters must be treated separately, and that the composition of individual plants must be tested, not by their ancestry, but by their progeny, for if this is done the process of selection is enormously simplified.

Mr Lock's book should be read by all interested in these matters; it is interesting throughout, generally clearly written, and has a good index and a glossary of technical terms for those who have no great acquaintance with the subject. And it has the further advantage of giving a great deal of information in a very small space.

VOLUME II DECEMBER, 1907 PART III

SOME AIR TEMPERATURE READINGS AT SEVERAL STATIONS ON SLOPING GROUND.

BY ROBERT S. VINSON AND EDWARD J. RUSSELL, D.Sc. (Lond.).
(South-Eastern Agricultural College, Wye.)

IT is well known that the mean temperature falls as the elevation increases, and partly on this account certain crops cannot profitably be produced on high land. But it is also recognised that low lying land is subject to lower minimum temperatures, and is therefore more liable to frost[1], than land higher up, and low situations are consequently avoided by fruit and early potato growers. Few actual temperature readings have, however, been published.

We have recently been making an experimental study of the phenomena of spring frosts, and have taken a series of temperature readings at several stations on a slope about a mile and a half long with a total difference in altitude of 580 feet. The slope is on the North Downs, which here run in a S.E. and N.W. direction; away to the S., S.E. and S.W. stretches a large plain. There are eight stations in all, one on the top, two on the banks of the river Stour at the bottom, and the other five come in between; the series form a somewhat irregular line running E. and W. in the positions shown in the following table.

TABLE I.

Position and description of stations.

No. of station	Height above sea level in feet	Height above river at station 7	Distance from station 7	Situation
1	675·21	581·69	7322·5	At the top of the Downs
2	285·34	191·82	5284	Poor pasture field
3	232·34	138·82	4126	Young corn crop
4	174·37	80·85	2691	Uncropped ground; somewhat sheltered from the north by a plantation
5	119·34	25·82	558	Pasture land
6	97·00	3·47	196	,,
7	94·52	1·00		River bank, confluence of river and ditch
8	97·00	3·47		River bank, straight part

[1] Theophrastus mentions this fact (lib. v. c. xx.) and also Pliny (lib. XVIII. cc. 69, 70); other old references are given in Boussingault, *Chimie Agricole*, II. 378, and in Warington, *Physical Properties of Soil*, pp. 184 *et seq.*

Stations No. 7 and 8 are intended to find what effect is produced by running water; No. 7 is much more surrounded than No. 8. The river is here 40 ft. wide and very slow. Mr R. S. Biscoe kindly determined the altitudes and distances.

At each station readings were taken at 6 inches and at 2 metres above the ground: they were started on April 14th and had to be discontinued on May 31st. There is a little uncertainty about some of the readings at 2 m., because strong winds shook the thermometers, and at times readings could not be taken but had to be calculated from the results obtained at the stations above and below. The average values used here are probably only slightly affected. Maximum and minimum temperatures were read at stations 1 and 6, but at the other stations minimum readings only were taken; the thermometers were fixed on posts in the open and had no protection whatsoever. All the thermometers used were checked against a standard thermometer with a Kew certificate.

Minimum Readings.

The means of all readings are given in Table II.

TABLE II.

Average minimum temperatures, degrees C., April 14th to May 31st, 1907.

							River bank	
No. of station	1	2	3	4[1]	5	6	7	8
Minimum temperature at 6″ above ground	3·4	3·2	3·3		2·6	2·2	3·3	3·0
,, ,, 2 m. ,,	3·8	3·4	3·6		3·2	3·2	3·1	3·1

Two distinct phenomena are here involved, the effect of elevation and the effect of water on temperature. Beginning with the former of these it will be noticed that the ground temperature reaches a maximum at the top station, and falls with decreasing altitude to a minimum at No. 6. The temperatures 2 metres up are somewhat higher than the ground temperatures, especially at Nos. 5 and 6. There is the same fall with decreasing altitude, but the gradient is much less marked, and at the four lower stations substantially the same readings are obtained.

[1] Readings spoiled by wind on several nights, and therefore omitted from this Table.

The effect of water will be discussed in detail later on, it is very strikingly shown at No. 7, where the ground temperature is a degree higher than at No. 6, although the two stations are less than 200 ft. apart and lie in the same field. At 2 metres the effect no longer shows.

These phenomena occur to a marked extent on still nights.

TABLE III.

Average minimum temperatures, 11 *still nights during experimental period.*

							River bank	
No. of station ...	1	2	3	4	5	6	7	8
Minimum temperature 6″ above ground ...	2·8	2·0	2·2	1·9	·6	0	1·5	·9
,, ,, 2 m. ,, ...	3·3	2·5	2·7	2·6	1·3	1·2	1·3	1·4

The difference in ground temperature at stations 1 and 6 has now increased to nearly 3°, and the warming effect of the river is well seen. At 2 metres the temperature is a shade lower at No. 6 than elsewhere, but there is very little difference between stations 5, 6, 7, and 8. These gradients are plotted in Fig. 1; it will be observed that they run parallel as far as station 5.

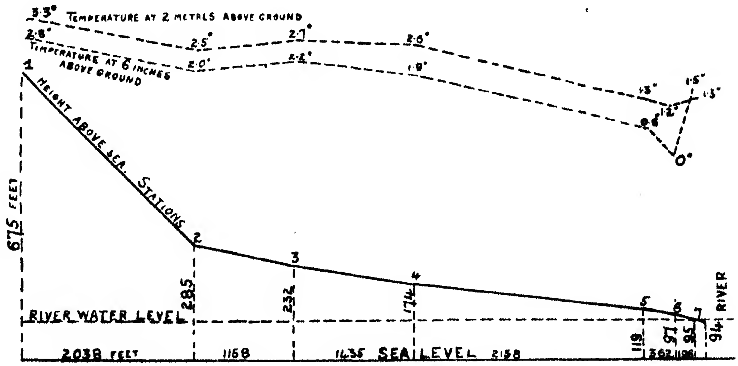


FIG. 1. Temperature gradients on still nights.

The usual explanation of the coldness of the lower ground on still nights is that as the temperature falls the layer of air near the ground is cooled, increases in density and tends to roll down the slope and collect on the plains or in the valleys. Here it displaces the warmer

air, which, being lighter, rises and flows over the upper slopes from whence the cold air has come, keeping these slopes at a higher temperature than the lower ones. This explanation is quite in accordance with all our results.

It is not only on still nights, however, that these effects are produced. We also observed them when there was a south or south-east wind, whilst the reverse effects were seen when the wind came from the north or north-east. We are not prepared to explain this result, but it is probably significant that the south and south-easterly winds blow over the plain and travel up the slope in the same direction as the drift of warm air. Apparently the downward flow of cold air near the ground is not prevented. There might, of course, be a greater cooling of the ground in the valley than on the hill, but this is very unlikely; the moist valley soil probably cools less quickly at night than the dry hill soil. Another important point is that the higher stations are naturally more exposed to the wind, and show the warming of the south wind, and the cooling of the north wind more than the less exposed stations lower down.

TABLE IV.

Average minimum temperatures, nights when wind is blowing.

No. of station	S. or S.E. wind blowing up the slope. Average of 7 nights		N. or N.E. wind blowing down the slope. Average of 6 nights	
	6″ above ground	2 m. above ground	6″ above ground	2 m. above ground
1	6·0	6·2	0·9	0·9
2	5·8	5·5	1·2	1·3
3	5·6	5·8	1·4	1·6
4	5·4	5·8	1·9	2·1
5	4·3	5·6	1·3	1·3
6	3·5	5·0	1·6	1·8
7 (River bank)	5·4	5·1	2·5	2·1
8 (River bank)	4·4	5·0	2·0	1·6

Closely connected with the difference in temperature at stations 1 and 6 is the difference in reading between the thermometers placed at 6″ and at 2 m. On still nights, and with a S. or S.E. wind, the temperature at 6″ is from ·2° to 1·5° lower than at 2 m., while with a N. or N.E. wind the difference is much less, and vanishes at stations 1, 2, and 5.

Effect of the river.

It has already been pointed out that stations 7 and 8 on the river are always warmer than station 6, 196 feet from the edge, but in the same field. Station 7, at the point where a ditch flows in, is almost surrounded by water, and shows a higher temperature than station 8, which is on a straight part. The warming effect is probably due to the high specific heat of water and the absorptive power of its vapour preventing a great fall in temperature during the night; it is only seen near the ground, and disappears at 2 m. The readings at 6 inches are given in Table V.

TABLE V.

Average minimum temperatures at and near river bank.
6″ above ground.

	Station 6, 196 ft. away from river	Station 8, on river bank, straight part	Station 7, on river bank, confluence of river and ditch
Minimum, all nights	2·2	3·0	3·3
Excess on river bank		·8	1·1
Minimum, still nights	0	·9	1·4
Excess on river bank		·9	1·4
Minimum, nights with S. or S.E. wind	3·5	4·4	5·4
Excess on river bank		·9	1·9
Minimum, nights with N. or N.E. wind	1·6	2·0	2·5
Excess on river bank		·4	·9

It should be noticed that the river is only 40 feet wide.

We cannot say how far from the bank the warming effect is perceptible, but practical men have long since recognised that fields and gardens near a river or a large mass of water suffer less from frost than those further away. Instances of this protective action can be found all along the north Kent coast. The Isle of Grain is less liable to frost, and therefore more suited to early potatoes, than the Hundred of Hoo, situated a little further from the water. Fruit close to the coast suffers less from frost than that a little inland. It is noticed in Worcestershire that gardens within 50 yards of the river Avon suffer less than gardens at a greater distance, whilst in California the influence of a river is so well recognised that land along the bank, and particularly at a bend, where the protective effect is intensified, is considered to be more valuable for certain fruits than land elsewhere.

On a slope like the one under consideration three zones can be distinguished, one near the river somewhat protected against frost, a second further away but on the low ground, and liable to frost, a third still further away on the high ground much less liable to frost. These zones are shown diagrammatically in Fig. 2; their relationships are important to the fruit and early potato grower in spring, and to the gardener in autumn.

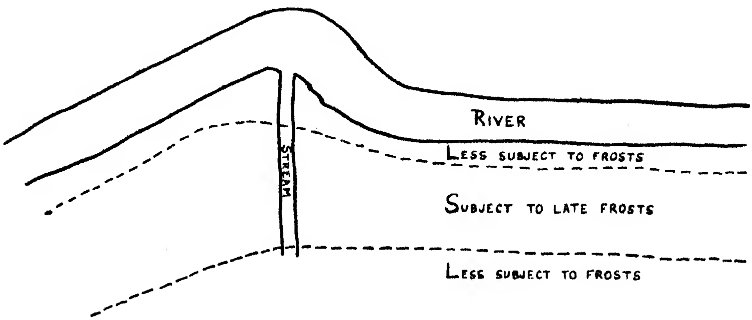


FIG. 2.

Maximum temperatures and temperature amplitudes.

Maximum readings were taken at stations 1 and 6, and the results show that during the day the temperature in the valley is higher than on the hill and in consequence the temperature amplitude is nearly 4° greater.

TABLE VI.

Average maximum and minimum temperatures, April 14th to May 31st.

	Station 1, top of slope	Station 6, bottom of slope	Excess at 6
Maximum	14·3	16·7	2·4
Minimum	3·4	2·2	1·2
Total variation ...	10·9	14·5	3·6

The fact that low land is colder by night and hotter by day than land higher up is well known and is also exemplified in the different character of plant growth in the two situations.

SOME OBSERVATIONS ON "SWOLLEN HEAD" IN TURKEYS.

By G. S. GRAHAM-SMITH, M.D.

University Lecturer in Hygiene, Cambridge.

THE following observations relate to a disease in turkeys, colloquially known as "swollen head," characterised by enormous swellings under the eyes and discharge from the nostrils, which was recently prevalent in some parts of the Eastern Counties. The disease appeared to be confined to turkeys, and was not communicated by them to fowls and ducks with which they mixed. Only turkeys sent to the laboratory were examined, and no opportunity occurred of making an inspection of the birds on the farms on which the disease was prevalent. From the reports of those who had to deal with the birds it was evident that when once introduced the disease spread rapidly. Usually the swellings are so large that the birds are unable to see their food, and unless artificially fed become very thin, but if artificially fed may remain in good health. Sometimes, however, a considerable mortality was produced. In some instances the owners opened the swellings and expressed the gelatinous contents, and washed out the infected cavities with antiseptic solutions. These measures produced some temporary alleviation of the symptoms, and allowed the birds to feed naturally for a day or two. The original condition, however, soon recurred.

Up to the present time very few investigations seem to have been made on the pathology of the lesions or the cause of the disease. Dodd (1905) appears to be the only observer who has described, under the name of "Epizootic pneumo-enteritis of the turkey," a disease in which similar swellings occur. In his cases, however, the swellings were accompanied by marked lesions of the lungs and intestines. In the present instance no lesions of the lungs or intestines, except dilatation of the coeca, in a few cases, were ever found, nor were the bacilli described by this observer ever recovered from the heart's blood or

organs. It is, therefore, possible that two diseases characterised by swellings under the eyes may affect turkeys, or that in more severe epidemics lesions of the internal organs occur which are lacking in the less severe types.

The present investigations consisted of (1) observations on naturally infected turkeys, including bacteriological examinations of the exudates during life and of the affected parts, blood and organs after death; and (2) the inoculation of healthy turkeys with pure cultures of the predominating bacteria found in the naturally infected birds, and with emulsions of the exudates of diseased birds.

As a result of these experiments it has been shown that the disease does not always follow the same course, and that it can be communicated to healthy turkeys in various ways during its different stages. Mainly owing to the limited material and accommodation no specific organism has, however, been isolated, and the cause of the disease has not, therefore, been determined.

It is hoped, however, that these observations in spite of the lack of definite conclusions may be of assistance to those who take up the study of this interesting and economically important disease.

Anatomy. In the turkey's head there is a large cavity, externally covered by skin, which surrounds the lower part of the orbit, and extends forwards almost up to the nostrils. This is a dilatation of the naso-pharyngeal system of air sacs, and communicates with the nasal cavity by a very narrow passage, through which it is filled by air. For the sake of convenience it is here described as the *infra-orbital sac.* Its dimensions and relations are best appreciated by reference to Plate III, fig. 3.

The naturally acquired disease.

Turkey No. 1. (Old Cock.) The bird showed large fluctuating swellings under both eyes. Commencing immediately in front of the eye at the level of the upper lid the swelling extended forwards almost up to the nostril and backwards under the eye to a point about a quarter of an inch behind the palpebral opening. It was most marked in front of the eye preventing the bird from seeing objects nearly in front of it, but also formed a considerable mass under the eye preventing the latter from being completely opened. The swellings were exactly similar in shape and extent on both sides.

A gelatinous fluid slowly exuded from the nostrils which the bird wiped off by passing the beak across the feathers of the back. These

feathers consequently became coated with the exudate and stuck to one another. Some frothy material was occasionally found on the surface of the eye.

The bird found considerable difficulty in feeding, as when the head was lowered the swellings in front of the eyes increased, and the bird was unable to see the dishes containing food, and in fact frequently failed to pick up any, often pecking the floor in the neighbourhood of the dish.

He showed no other evidence of disease and appeared to be in good health.

This bird was kept under observation for nearly two months. During the first two weeks the contents of the swellings were daily expressed through openings which had previously been made. Within a few hours, however, they had again filled up. During the rest of the time it was artificially fed. No difference was noticed in the size or consistency of the swellings and the general health was well maintained. At the end of this time the bird was killed and a careful autopsy made.

Autopsy. All the organs appeared to be normal, including the pericardium and intestines. Cultures on various media from the heart's blood, liver and lungs remained sterile. Sections of the organs showed no pathological lesions.

The swellings were found to be caused by the distension of the infra-orbital sacs by gelatinous, semi-transparent fluid like egg albumen containing some white flocculi. On the left side a small mass of yellowish material, like inspissated pus, was found in the anterior part of the cavity. The nasal cavities also contained some gelatinous fluid, and the mucous membrane appeared somewhat swollen, but was not markedly congested.

No motile organisms or protozoa were found in fresh films. Stained film preparations and cultures were made from the nasal exudate during life and from the gelatinous fluid after death. In both cases the fluid showed small numbers of cells and large numbers of bacteria. The great majority of these belonged to one species, which is described under the name of Bacillus A.

Bacillus A. In film preparations these bacilli were scattered over the field singly or in groups the members of which were arranged at all angles. Two individuals were occasionally found end to end but chains were never observed. The bacilli stained well by Gram's method and by methylene blue. In most cases the ends stained more darkly than the centre, and a light band in the middle was

a conspicuous feature. They all showed rounded ends, and the majority were slightly curved. Considerable variation in size was noted, a few being long, the majority of medium length, and a few short. All showed slight irregularities in width, so that they were never cylindrical, and many showed clubbed ends. They are non-motile and no spores are formed.

In cultures on serum made directly from the gelatinous exudate numerous colonies of this bacillus developed, together with a few colonies of other organisms, probably accidental impurities. In pure cultures it exhibited the following characters.

Serum. After 24 hours incubation at 37° C. small, smooth, circular, moist, dome-shaped, colonies of greyish colour are formed, which show a tendency to coalesce. The bacilli are of medium length, slightly curved, often slightly clubbed, and show a central light band and darkly staining rounded ends. A few long segmented forms are also present. No polar bodies are formed. After 48 hours growth the colonies are larger, and many have coalesced, and long segmented forms of the bacilli are more numerous. *Agar.* After 24 hours growth at 37° C. small, round, flat, grey, almost transparent colonies are formed. The central portion is more opaque than the rest and is surrounded by a very thin grey zone with a granular surface. After 48 hours the colonies increase in size but are similar in shape. Later, however, the colonies lose their rounded form, and throw out spike-like projections. Many long segmented bacilli are found after 48 hours growth. Growth occurs slowly on *gelatin* and the medium is not liquefied. On *potato* after 24 hours growth a slightly yellowish continuous growth is formed, which later becomes decidedly yellow in its thicker portions. Most of the organisms are rounded and appear like diplococci, but a few of the larger forms are to be found. *Broth* cultures grown at 37° C. at first show a very slight surface film, and slight turbidity, and a granular deposit. Later the medium becomes clear. The organisms are long, segmented and clubbed. The reaction of *glucose-broth* is not altered. The bacilli are *non-pathogenic* to guinea-pigs in large doses intra-peritoneally.

Turkey No. 2. (Adult hen.) The right side of the head was normal. On the left side there was a large swelling exactly corresponding in its situation to the swelling in turkey 1. It differed, however, from the latter in being distinctly hard, and in being covered by dry and brown, instead of normal, skin. Fluctuation could only be obtained with difficulty (Plate III, figs. 1, 5). Clear gelatinous fluid exuded from the left nostril, and, as in the case of turkey No. 1, the feathers on the back were coated with this material. The bird was rather thin, but appeared to be in good health and took its food well.

In the course of time the swelling became larger and harder. Five months after the bird was received the swelling was enormous, entirely preventing the bird from using the left eye. The palpebral fissure, however, remained widely open owing to the lower palpebral conjunctiva being forced through it by the accumulation of material under it (Plate III, fig. 2). Shortly afterwards this portion of the con-

junctiva ulcerated, and a considerable amount of yellowish, foul-smelling material was discharged in large masses, and so diminished the swelling that the cornea again became visible. Up to this time the eye itself was apparently uninjured, the bird fed well and the general health seemed unaffected.

Within the next month the swelling again increased in size, and the bird refused to eat, and becoming very thin and weak was killed.

Autopsy. The body was extremely emaciated, but no macroscopic or microscopic lesions were found in any of the cavities or organs. Cultures from the heart's blood and several of the organs remained sterile. On the right side the eye and infra-orbital cavity were normal.

On the left side the whole of the infra-orbital cavity was filled with a dense, laminated, yellow cheesy mass of foul-smelling material, which also covered the anterior surface of the eye. At one place the cornea had become ulcerated, and similar material filled the anterior chamber. This lesion was evidently very recent. The left nasal cavity was filled with clear gelatinous fluid containing small flakes, but the walls showed no inflammatory changes. The mouth was normal.

Sections made from fragments of the material discharged during life showed that it was mainly composed of cells in various stages of degeneration. In the central portions a few pale staining nuclei only were seen imbedded in granular débris, but nearer the edges the nuclei and cells were more distinct. The latter are round and possess large round nuclei, and closely resemble the cells seen in the gelatinous exudate of turkey No. 1. No fibrin was found. In the central portions groups of bacteria were occasionally seen, but near the surface the bacteria were present in enormous numbers, lying singly or in large masses. In sections stained by Weigert's method many of the bacteria were well stained. Sections, obtained at the autopsy, prepared from masses of material filling the cavity were similar in all respects, except that the Weigert staining bacilli were much less abundant.

Bacteria. Fresh and dried film preparations and cultures were made at various times from the discharge during life and from the contents of the cavity after death. Films of the *gelatinous exudate* from the nostril during life showed numerous cells, the majority with large round or oval nuclei, mixed with other cells of various kinds. In these films bacteria were very numerous. The Bacillus A previously described and another later described as Bacillus C were occasionally seen, but the species B was much more common. A few large thick bacilli were also seen and many small cocci. Cultures showed Bacillus B to be the predominating organism. Film preparations from the *yellow material* discharged during life showed the same cells as the fluid but in much greater numbers. The great majority were undergoing degenerative changes. Bacteria were present in great numbers including cocci and the bacilli mentioned. Cultures on serum gave very large numbers of Bacillus B, which possesses the following characters.

Bacillus B. On *serum* after 48 hours growth at 37° C. small, round, slightly yellow, granular colonies appear situated in small depressions in the medium. Liquefaction does not occur on further growth. The organisms are non-motile, do not form spores, and have no definite arrangement in the field. They have rounded or slightly pointed ends. Some are of medium length and slightly curved, and others are long and markedly curved. Considerable enlargement of one or both extremities is very common except amongst the shortest forms, and some of the larger forms show enormous clubbed ends.

They stain well by methylene blue and also by Gram's method. By the former method all show differential staining of the protoplasm. The shorter forms show a central oval darkly staining area and pale extremities, while the long forms frequently show two or three of these dark areas. On *agar* after 24 hours growth at 37° C. round, raised, smooth, moist white colonies without distinctive features are formed. Most of the bacilli have marked swellings at one or both ends, almost all are more or less curved and have nodosities at various points, and some are distinctly segmented. These features are much exaggerated on further growth. No polar bodies can be demonstrated. On *potato* the growth is at first invisible, but becomes apparent after six days' incubation. Large distorted involution forms are rapidly developed. In *broth* no surface film is produced, but a good growth occurs in the form of a granular sediment while the medium remains clear. Neither acid nor gas is produced in media containing *glucose*, *saccharose*, *maltose*, *lactose*, *laevulose*, or *glycerine*. On *gelatin* growth occurs slowly and the medium is not liquefied. No change is produced in *milk*. The organism is *non-pathogenic* to guinea-pigs in doses of 2 c.c. intra-peritoneally and subcutaneously.

Films and cultures from the material obtained at the autopsy showed large numbers of bacteria. These differed in species, however, from those previously obtained, since very few bacilli belonging to types A or B were seen or cultivated. *B. pyocyaneus* was isolated and appeared to be typical in all respects including pathogenicity to guinea-pigs. The majority of bacilli belonged to the species C about to be described.

Bacillus C (Plate III, fig. 7). In film preparations from the original cheesy material the organisms appear as medium length, rather wide rods, with rounded ends. They vary greatly in length, and stain poorly but evenly with the ordinary dyes, and do not retain Gram's stain. On *agar* after 24 hours growth at 37° C. white, flat, smooth, round colonies are formed of somewhat slimy consistency. In smear preparations made from these colonies the bacilli tend to stick together in masses. The bacilli are medium length, rather wide rods with rounded ends which stain evenly. Occasionally long forms occur, and sometimes short almost round forms. They are non-motile and no spores are formed. This bacillus grows well on *serum* forming medium sized, whitish, round, soft colonies, which rest in small depressions in the medium. Liquefaction does not occur. The bacilli resemble those from agar cultures. Good growth occurs on *gelatin* and the medium is not liquefied. The colonies are small, irregularly rounded, granular and semi-transparent. The organisms are short and uniformly staining. *Broth* at first shows marked turbidity but the growth soon settles in the form of a thick, whitish, flocculent deposit. On *potato* a copious white slimy growth is produced in 24 hours. Subsequently the growth becomes very abundant, but is never coloured. Media

containing *glucose*, *lactose*, *maltose*, *mannite*, and *dextrin* all become markedly acid. *Milk* is made acid and firmly clotted within 48 hours. Guinea-pigs die within 18 hours after intra-peritoneal inoculation of 1 c.c. of broth culture. At the autopsy a purulent peritonitis is found with large numbers of organisms in the fluid.

Turkey No. 3. (Young hen.) Both sides were affected with soft fluctuating swellings exactly similar in position and size to those of turkey No. 1. There was also a considerable amount of glairy discharge from both nostrils, and the feathers of the back were plastered together with this material. The bird was very thin, and owing to an ulcerated condition of the upper mandible kept its mouth slightly open.

Cultures made from the nasal discharge showed *B. pyocyaneus* and Bacilli B and C. During the first three weeks' observation the swellings became gradually smaller, and after four weeks that on the left side had completely disappeared and the other was scarcely visible. The discharge from the nostrils, however, continued and the bird looked very thin and ill. A few days later the swellings again became visible, but soon again disappeared. At the end of 8 weeks' observation the bird appeared very thin and had great difficulty in feeding owing to the condition of its beak. It was then killed.

Autopsy. Except in the case of the coeca no lesions were found in any of the organs or cavities of the body either macroscopically or microscopically, and cultures from them remained sterile. The distal extremities of both coeca were greatly distended by frothy gelatinous-looking material with a foetid odour. Small masses of yellow coagulum were sticking to the walls. Amongst other organisms Bacillus C was isolated from them.

Both infra-orbital cavities contained fluid similar to that obtained from turkey No. 1. Cultures showed a few colonies of *B. pyocyaneus* and Bacillus B, and a considerable number of Bacillus C. Cocci and other organisms were also present.

Turkey No. 4. (Young hen.) This bird showed no signs of the typical swellings, but suffered from difficulty in breathing, and the nostrils were stopped up by hardened secretion and the eyes frequently filled with frothy fluid. It was then very thin and weak, and was considered to be in the last stages of the disease. After a few days' observation it was killed and an autopsy made.

Autopsy. The trachea contained three worms (*S. trachealis*), and the tracheal mucus was full of their ova. All the other organs appeared to be normal and cultures from them remained sterile. The infra-orbital cavities contained practically no fluid, and only a few cocci

appeared in cultures. From the tracheal mucus, however, an organism apparently identical with Bacillus A was cultivated.

Experiments on turkeys.

Turkey A. (Young cock.) Attempts were made to infect this bird by several methods: (1) 1 c.c. of gelatinous material from turkey No. 2 was injected into the right infra-orbital cavity, and some of the same material was introduced by means of a swab into the left nasal cavity and into the mouth. The bird was carefully watched for four weeks but developed no symptoms of the disease. (2) At the end of a month a broth culture made directly from gelatinous material and incubated for 48 hours was mixed with the food. During the next four weeks the bird remained in perfect health. (3) Ten lice from a naturally infected turkey were now placed on him, and again the bird showed no symptoms for a month. (4) During the next week his food was contaminated with the excrement of a diseased turkey. The bird showed no symptoms during a month's observation. (5) The bird was now placed for six weeks in the same cage with an infected turkey without showing any signs of the disease. (6) Finally 2 c.c. of cheesy material from the eye of turkey No. 2, emulsified in salt solution, was inoculated into the right infra-orbital cavity. The next day a slight swelling appeared, which increased considerably during the next two days. In five days the swelling was of the same size and consistency as the swellings observed in turkey No. 1, and caused partial closure of the eye. A considerable quantity of gelatinous fluid escaped from the right nostril, and this could be increased by pressure over the swelling. Smears and cultures from this material showed numerous organisms including Bacilli B and C. During the next month the swelling became distinctly harder, and the discharge from the nostril more abundant, so that it constantly dripped from the beak, and plastered down the feathers of the back where the beak was wiped. Within the next three days, however, the discharge completely ceased, and the nostril was occluded by coagulated material. A week later the bird was killed and examined after being under observation for 23 weeks.

Autopsy. All the organs and cavities appeared normal, and microscopic sections showed no lesions. Cultures from the heart's blood, lung, and liver remained sterile. The left side of the head was normal, and no changes were noticed in the mouth or left nasal cavity.

The inoculated infra-orbital cavity contained a yellow mass forming a perfect cast (Plate III, fig. 6) of the cavity and its ramifications. The membrane lining the cavity was rough and injected, but in no place intimately adherent to the cheesy material. The eye was unaffected. The mucous membrane of the right nasal cavity looked swollen, and small masses of yellow material were found in the crevices.

Smears from the yellow mass showed many cells, mostly undergoing degeneration, and numerous bacteria, including Bacilli B and C, and a few streptococci and other organisms. Cultures showed a few colonies of Bacillus B and numerous colonies of Bacillus C.

Sections of the nasal mucous membrane showed some dilatation of the vessels and many inflammatory cells. Sections of the lining membrane of the infra-orbital cavity showed that, except in some of the recesses, the epithelium had disappeared and was replaced by granulation tissue. The subcutaneous tissue between the skin and the infra-orbital cavity contained much new formed fibrous tissue, and was infiltrated with inflammatory cells. The bacteria had not, however, penetrated deeply.

Turkey B. (Hen.) Crushed cheesy material from turkey A was inoculated into the right infra-orbital cavity. In three days a slight swelling appeared which gradually increased in size. After 18 days the swelling was still of moderate size and soft, and a gelatinous discharge had developed from the nostril. In the next week the latter increased in amount and cultures showed that it contained some cocci, a few colonies of *B. pyocyaneus* and Bacillus C, and a bacillus described as D in very large numbers. During the next 15 days the swelling gradually became harder, and the bird took its food badly and became weak. On the 51st day of the experiment the bird was found dead.

Autopsy. There seemed to be slight dilatation of the intestinal vessels, but all the other organs appeared healthy. Microscopic sections showed no lesions, and cultures from the heart's blood, pericardium, and liver remained sterile. Cultures from the lungs showed a few colonies of the Bacillus A type. The left side of the head was normal (Plate III, fig. 3). The right infra-orbital cavity contained foul-smelling cheesy material similar to that found in other cases (Plate III, fig. 4). The eye was not affected. Cultures from the affected cavity showed numerous colonies of Bacillus C and smaller numbers of Bacillus D and *B. pyocyaneus.* Cultures from the mouth showed the same organisms as well as various other bacilli and cocci.

Bacillus D. In smear preparations of the gelatinous material these bacilli were found in groups usually composed of 3—10 individuals, but occasionally containing as many as 40—50. Most of the specimens were curved, showed well marked segmentation, and well defined polar bodies (Plate III, fig. 8).

On *serum* after 24 hours growth at 37° C. the colonies are small, round, yellowish, and dry looking. After three days' incubation the colonies become very large. The centre is much raised and deep yellow in colour and is surrounded by a flat pale yellow zone. The whole colony has a very granular appearance. If the colonies are crowded together they coalesce to some extent to form a film which appears to have a wrinkled surface owing to the irregularities in height of the component colonies. The bacilli bear a remarkable resemblance to true diphtheria bacilli. They are of medium length, with rounded ends, and are usually slightly curved. Clubbed extremities are common and many have irregularities in their length. Some branching forms were seen. They retain Gram's stain, show polar bodies, terminal and central, with Neisser's stain, and differential staining of the protoplasm with methylene blue. In some dark and light bands alternate causing a segmented appearance, whilst in others these areas are irregularly placed. They are non-motile, do not form spores, and have no characteristic arrangement. On *agar* large, white, granular, irregular, dry-looking, heaped up colonies are formed, which after a few weeks' growth increase in size and become wrinkled. The bacilli are shorter than when grown on serum and many coccus-like forms can be found after 48 hours growth. On *potato* large, yellow, dry-looking, discrete colonies are produced. At first the bacilli are like those from serum cultures, but later large irregular involution forms are common. Good growth occurs on *gelatin* and large, dull-yellow, granular colonies are formed. The medium is not liquefied. In *broth* a whitish wrinkled film is produced on the surface, and a yellowish flocculent deposit. Acid is produced in media containing *glucose*, *galactose*, and *laevulose*, but not in media containing *lactose* and *glycerine*. This organism is *non-pathogenic* to guinea-pigs.

Turkey C. (Young bird.) The right infra-orbital cavity was inoculated with ·5 c.c. of gelatinous exudate from the nostril of turkey B. No symptoms appeared during three weeks' observation. Later this bird was inoculated with 1 c.c. of a 48 hours broth culture of Bacillus A, derived from the lung of turkey B, without effect. Some weeks later the bird was placed in the same cage as a naturally infected bird. After eight days a slight swelling appeared on the right side, and within the next 14 days considerable swellings had developed on both sides. During the next month the swellings gradually decreased and finally almost disappeared. The bird was then killed.

Autopsy. All the organs appeared to be healthy, and cultures from them remained sterile. The infra-orbital cavities only contained very small quantities of a milky fluid from which Bacillus C and *B. pyocyaneus* were cultivated. Although the coeca showed no lesions, Bacillus C was cultivated from them.

Turkey D. (Young bird.) The right infra-orbital cavity was inoculated with ·5 c.c. of a broth culture of a recently separated culture of Bacillus C. No symptoms were observed. Several weeks later the bird was placed in the same cage as a naturally infected bird. Within a week it developed considerable swellings on both sides, which gradually increased for about a month, but almost disappeared within the next month. In the ninth week of observation the bird suddenly died.

Autopsy. The bird was rather thin, but the only lesion found was distention of the coeca. Both infra-orbital cavities contained small quantities of cheesy material from which Bacillus C was cultivated. The same organism was isolated from the contents of the coeca.

Turkey E. (Young bird.) Gelatinous nasal exudate was placed in both nasal cavities and in the mouth on three consecutive days without effect. Some weeks later the right infra-orbital cavity was inoculated with a recent culture of Bacillus D without producing any symptoms. Several weeks later some gelatinous nasal exudate was placed in the right nostril. In eight days there was a discharge from the nostril, which continued for 15 days, when a slight swelling developed. Soon after the other side became swollen, and 40 days after the beginning of the experiment both sides were considerably swollen. The bird was then killed.

Autopsy. All the organs appeared to be healthy and cultures from them remained sterile. The right infra-orbital cavity contained very little gelatinous fluid, and the left similar fluid and some cheesy material. Cultures were overgrown by a film-forming organism.

Turkey F. (Young bird.) ·5 c.c. of a recently isolated culture of *B. pyocyaneus* was inoculated into the right infra-orbital cavity without producing any effect. Some weeks later 1 c.c. of an emulsion of the cheesy material from turkey B was inoculated into the left infra-orbital cavity. A very slight swelling developed which gradually disappeared.

Turkey G. (Young bird.) This bird lived for a considerable time in the same cage with turkey B without developing any symptoms. Some weeks later some gelatinous nasal discharge was given to the bird on pieces of lettuce. A week later a discharge from the nostril appeared, which continued for about five weeks, when both sides became slightly swollen. Shortly afterwards the discharge ceased, and the swellings gradually disappeared.

Turkey H. (Young bird.) This bird was inoculated with ·5 c.c. of

gelatinous material into the left infra-orbital cavity. After five days a distinct swelling was evident, and a few days later discharge appeared at the nostril. Soon afterwards the other nostril began to discharge, and this was followed by a swelling. Within a month there were large, soft, fluctuating swellings on both sides. A fortnight later the bird died.

Autopsy. With the exception of the coeca all the organs and cavities of the body appeared to be healthy and cultures from them remained sterile. Both the coeca showed some dilatation at their distal ends. Considerable quantities of gelatinous fluid were found in both infra-orbital cavities. Cultures from the coeca showed numerous bacteria of various kinds, and amongst others Bacillus C was isolated. Cultures from the gelatinous fluid of the infra-orbital cavities showed very few bacteria and none belonging to the species described.

Experiments on other birds.

Intra-muscular injection of some gelatinous fluid into a pigeon produced no effect, and several experiments including inoculations of pure cultures of Bacilli B, C, D, and *pyocyaneus* and gelatinous and cheesy material into chickens were all negative.

Summary.

In experimentally infected birds a clear viscid discharge from the nostril is sometimes the first symptom noticed, and may occasionally be the only one for a long time (turkey E). This is followed by a soft fluctuating swelling under one or both eyes. After the development of a well-marked swelling, the course of the disease may vary both in naturally and experimentally infected birds. In some cases the swelling gradually diminishes and finally disappears (turkeys 3, C, D, F, and G), in other cases the soft fluctuating swelling persists for considerable periods (turkeys 1, E and H), and in others the swellings become hard and the gelatinous contents are replaced by cheesy, foul-smelling material (turkeys 2, A and B). The latter condition may be due to secondary infection. Sometimes the disease only attacks one side leaving the other quite normal. Death occurred in some instances, but could not be definitely attributed to the disease.

Careful autopsies made on all the turkeys which died or were killed failed to show any gross or microscopic lesions of the cavities or principal organs of the body. Cultures taken from the heart's blood, organs and

cavities all remained sterile, except in the case of a single culture from the lung of turkey B, which showed a few colonies of Bacillus A. In three instances apparently abnormal dilatation of the coeca was found, but even in these cases no indications of inflammatory reaction were noticed. In six birds no lesions of the coeca were noticed. In all cases, therefore, the disease was confined to the infra-orbital and nasal cavities.

The local conditions varied according to the character of the exudate filling the infra-orbital cavity.

In some cases, which were apparently recovering, only a small quantity of gelatinous material resembling egg albumen together with a few white flakes was found. In other cases in which the swelling was considerable large quantities of this material were present. Even in these cases very few signs of inflammatory reaction were noticed in sections of the walls of the cavities. In the cases in which whitish foul-smelling material was present the conditions were very different. The whitish-yellow firm mass completely filled the whole cavity, extending into the various recesses. In some instances it adhered firmly to the walls, but in one case could with some difficulty be removed as a perfect cast. Sections of the walls showed destruction of the lining membrane and extensive inflammatory changes. The nasal cavity on the affected side usually contained semi-gelatinous fluid resembling that found in the infra-orbital cavities in the milder cases, but in no instance was blocked by cheesy material. The mucous membrane often had a swollen gelatinous appearance, but never showed signs of acute inflammation. No lesions were ever found in the mouth.

Attempts were made to reproduce the disease by various methods, but of those tried more than once only one was uniformly successful. The disease was three times reproduced by means of the characteristic gelatinous material, once by its application to the nostrils, once by direct inoculation into the infra-orbital cavity, and once by feeding. On the other hand application to the nostrils failed once and direct inoculation twice. Inoculations with cheesy material were made three times and were successful on each occasion. Confinement in the same cage with a diseased bird was twice successful, and twice unsuccessful.

Contamination of the food with the excreta of a diseased turkey, and transference of lice from a diseased to a healthy bird were each tried, once unsuccessfully.

Inoculations into the infra-orbital cavities of healthy turkeys of

recent pure cultures of the organisms (Bacilli A, C, D, and *pyocyaneus*), present in large numbers in the exudate of diseased turkeys, all failed.

The experiments are neither sufficiently numerous nor conclusive to warrant any decided opinions on the cause of infection or the period of greatest infectivity. They seem to indicate, however, that the disease may be naturally contracted by contact with diseased birds during the gelatinous stage, by contamination of the nostrils with gelatinous material and by contaminated food. Experimentally the direct inoculation of cheesy material is the most certain method.

In spite of the negative results of the inoculations of pure cultures the relations of the predominating bacteria to the various stages are of considerable interest.

On several occasions fresh preparations of the various types of exudate were examined, but no motile bacteria or protozoa were noticed, and specimens stained by Giemsa's method did not show any organisms resembling protozoa.

In each instance one or at most two types of bacilli were present in such enormous numbers that without further investigation they might well have been regarded as the cause of the disease. Cultures were made from the fluid exuding from the nostrils of five diseased birds. Bacillus C was found four times, Bacillus B three times, Bacillus A and *B. pyocyaneus* twice each and Bacillus D once. Cultures from the gelatinous contents of the infra-orbital cavity of turkey 1 showed Bacillus A almost in pure culture, and from turkey 3 Bacilli B, C and *pyocyaneus* were obtained. Five sets of cultures were made from the cheesy material of various birds. All showed Bacillus C. Two showed Bacillus B also, one Bacillus D and three *B. pyocyaneus*.

In these 13 sets (7 turkeys) of cultures, Bacillus C was found 10 times (6 turkeys), *B. pyocyaneus* 6 times (4 turkeys), Bacillus B 6 times (3 turkeys), Bacillus A 4 times (2 turkeys), and Bacillus D twice (1 turkey). Moreover Bacillus C was isolated from the distended coeca of three turkeys, and the normal coeca of one turkey.

It is, therefore, seen that Bacillus C was almost constantly and *B. pyocyaneus* very frequently present. The other organisms were each present on at least one occasion in very large numbers.

The bacilli called A and B and more especially D seem to belong to the diphtheroid group. Organisms belonging to this group have been found by several observers in the mouths of healthy and diseased birds (Macfadyean and Hewlett (1900) in pigeons, Harrison (1901),

Guerin (1901), Graham-Smith (1904), and others in fowls). Similar organisms (*B. xerosis*) are found in the normal human conjunctiva and in those of some animals. In the human subject diphtheroid bacilli have been encountered in the throat, nose, ear, genital organs, and on the skin under various conditions. Considering the wide distribution of this group the occasional presence of members belonging to it in a lesion closely connected with the nose and eye is, therefore, not very surprising. They are, however, of considerable interest from two points of view. Firstly they occurred in some cases in such numbers, doubtless due to favourable conditions for multiplication, that without control observations and experiments they might well have been regarded as the cause of the disease, and secondly, in view of the diagnosis of true diphtheria in fowls and turkeys by some authors without sufficient bacteriological evidence, the extraordinary morphological resemblance of Bacillus D, and some examples of Bacilli A and B, to the true diphtheria bacillus demonstrates the necessity for careful cultural and animal experiments before a diagnosis of diphtheria in birds can be made.

B. pyocyaneus though capable of giving rise to various lesions seems often to be a harmless saprophyte. In these cases though pathogenic to guinea-pigs it did not appear to have any relation to the disease.

The case of Bacillus C is, however, different. It was almost always present in greater or lesser numbers, and was extremely pathogenic to guinea-pigs. On the other hand, in the one experiment in which a pure culture was injected, no effect was produced in a turkey. In estimating the value of this negative experiment the fact must be borne in mind that on two occasions gelatinous exudate applied to the nostrils, and on two occasions the inoculation of this material into the infra-orbital cavity, produced no symptoms, though subsequent experiments proved that under both conditions the disease could be reproduced in turkeys.

It is possible that the disease may be due to other bacteria or cocci, which were never present in very great numbers, or to ultra-microscopic organisms such as are known to give rise to some diseases.

The disease resembles that described by Dodd (1905), in being characterised by the presence of large fluctuating swellings under the eyes, but differs in the fact that the lesions are almost entirely local. Dodd observed thick creamy deposits in the mouth, consolidation and yellow foci in the lungs, and marked lesions of the intestines. One of

the breeders, a medical man, from whom he received some of his specimens, also observed very extensive lesions in the lungs. Dodd further found organisms of the fowl-cholera type in the gelatinous material, heart's blood and organs. Neither bacilli of this type nor the lesions just mentioned were ever encountered. Dodd's bacilli were pathogenic for pigeons, producing dry necrosis at the seat of inoculation, diphtheritic deposits in the mouth, congestion of the lungs and intestines and septicaemia. They were non-pathogenic for fowls, but produced local lesions in guinea-pigs and death in rabbits. He assumes that these organisms were the cause of the disease but made no experiments on turkeys.

In conclusion I wish to express my very sincere thanks to Mr T. B. Wood of the Agricultural Department for calling my attention to the disease and for supplying me with all the turkeys both healthy and diseased.

Conclusions.

(1) The characteristic fluctuating swelling is due to the accumulation of gelatinous material in the infra-orbital cavity.

(2) The swelling may disappear and the bird recover, or the swelling may, in spite of frequent washing out of the cavity, remain for months in the same condition, or the gelatinous material may be converted into cheesy, foul-smelling material. Death occasionally takes place whatever course the disease follows.

(3) With the exception of occasional distention of the coeca, which may have no connexion with this disease, the lesions are confined to the head.

(4) Bacilli of the diphtheroid group (A, B and D) are frequently found in the exudate, and a pathogenic organism (Bacillus C) is almost invariably present, often in very large numbers. Up to the present, however, the latter has not been definitely proved to be the cause of the disease. All attempts to reproduce the disease by means of pure cultures of these organisms failed.

(5) The disease can be reproduced most certainly by the inoculation of cheesy material into the infra-orbital cavity, but can also be reproduced by inoculating the infra-orbital cavities, or smearing the nostrils, or contaminating the food with the gelatinous exudate, or by close contact with the diseased birds.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

REFERENCES.

DODD, S. (1905). *Journ. of Comp. Pathol.* XVIII. p. 239.
GRAHAM-SMITH, G. S. (1904). *Journ. of Hygiene*, IV. p. 258.
GUERIN, C. (1901). *Ann. de l'Inst. Pasteur*, XV. p. 941.
HARRISON, F. C. (1901). *Report of Ontario Agr. College.*
MACFADYEAN, A. and HEWLETT, R. T. (1900). *Trans. Path. Soc.* LI. p. 13.

EXPLANATION OF PLATE III.

Fig. 1. Photograph of the left side of the head of Turkey No. 2 at an early stage of the disease when the swelling was soft. The swelling is mainly in front of the eye which is partially closed.

Fig. 2. Photograph of the left side of the head of the same turkey at a later stage. The swelling had by this time increased greatly in size and had become hard. The accumulation of cheesy material under the lower conjunctiva had forced it over the cornea to such an extent that the palpebral fissure was kept widely open. At this period the eye had not become involved.

Fig. 3. Photograph of a dissection of the left side of the head of turkey B to show the extent of the normal infra-orbital cavity.

Fig. 4. Photograph of a dissection of the right side of the head of turkey B to show the infra-orbital cavity filled with white cheesy material.

Fig. 5. Photograph of the upper surface of the head of turkey No. 2 to show the extent of the swelling on the left side. In order to secure a white background the bird's head was thrust through a piece of white filter paper. (Same stage as fig. 1.)

Fig. 6. Photograph of the cheesy contents of the right infra-orbital cavity of turkey A removed in the form of a cast. (Rather larger than the natural size.)

Fig. 7. Drawing of a smear preparation of cheesy material stained with methylene blue. Most of the bacteria seen belong to the species C. (Zeiss $\frac{1}{12}$ Oil. Imm. Oc. 4.)

Fig. 8. Drawing of part of a section stained by Weigert's method of some of the cheesy material from the right infra-orbital cavity of turkey B. Groups of Bacillus D are shown amongst the nuclei of the cells. Bacteria which do not stain by this method cannot be seen. (Zeiss $\frac{1}{12}$ Oil. Imm. Oc. 4.)

THE FLOCCULATION OF TURBID LIQUIDS BY SALTS.

By A. D. HALL, M.A. and C. G. T. MORISON, B.A.
The Rothamsted Experimental Station.

It has long been recognised that a trace of soluble salt will bring about the flocculation of the material suspended in a turbid liquid; and because of its bearing upon such practical matters as the clearing of drinking water, the deposition of silt at the mouths of estuaries, and the improvement of the texture of heavy soils, the process has received a considerable amount of investigation. It is probably related to the flocculation of colloids by similar reagents; the fine particles of clay which chiefly cause the turbidity of natural waters being composed of a hydrated silicate of alumina and other bases possessing properties akin to those of the colloids. Indeed Schloesing has proposed to call that portion of clay material which remains obstinately suspended in water, sometimes for days together, 'colloid clay,' as something distinct in kind from the other particles in the soil. However it is reasonable to suppose that the distinction between the 'colloid' clay and the rest is in the main one of size, the colloid particles being so small as to be beyond the limits of microscopic vision, just as the true colloids may be regarded as consisting of very large molecules, lying between the molecules which go into solution and the molecular aggregates that remain in water as suspended solids.

Various theories have been proposed to account for the phenomenon of flocculation (see Schulze, *Journ. Prac. Chem.* 25 (1882), 431; Picton and Linder, *Trans. Chem. Soc.* 67, 1895, p. 63; Whetham, "Theory of Solutions," *Phil. Mag.* v. 48, 474 (1899); Joly, *Compt. Rend. du VIII^e Congrès Géologique International*, 1900; Spring, *Rec. Trav. Chim. Pays Bas*, 1900, p. 222, which paper also contains a bibliography), but since they did not seem to accord with other observations on the behaviour

of saline solutions towards clay, the authors decided to re-examine the whole subject.

Since the flocculation of 'natant' material and the clearing of the turbid liquid is a question of degree, depending on such factors as the strength of the flocculating salt solution, time, temperature, &c., no absolute measure of flocculation can be obtained. Only a comparative method of experiment can be adopted, and much of the difficulty of correlating the work already done lies in the want of definition of what the author has meant by flocculation. For the present investigations a standard material was first prepared: this consisted of a purified kaolin from which the coarser particles had been separated by decantation in water; it would remain suspended in a column of water 7·5 cm. high for more than 24 hours and was made up of particles less than 0·002 mm. in diameter.

A stock of this having been prepared and suspended in a large bulk of water, 10 c.c. were withdrawn for each experiment after brisk shaking, a quantity that was found to contain 0·2219 gram. of kaolin.

The experiments were carried out in glass cylinders holding 200 c.c. of water to which the 10 c.c. of kaolin suspension were added together with a measured quantity of a standardised solution of the flocculating salt, the whole being then thoroughly stirred. The cylinders stood in an unheated room free from vibration or much change of temperature, and after a few hours, when clearing had sufficiently progressed, each jar was matched by eye with one or other member of a standard series. This standard series was made up each time of a number of cylinders in which the flocculation was effected by regular increments of calcium nitrate, so as to obtain concentrations lying between $n/40{,}000$ and $n/2500$. Thus by preliminary trials and by varying the concentration of the substance under examination it could be matched against one of the standard jars and could be brought into a comparative numerical scale indicating its flocculating power, on the assumption that this flocculating power is inversely proportional to the amount of substance required to produce the standard effect. Thus if a concentration $=n/2000$ normal of a substance A is required to produce the same result as a concentration of $n/10{,}000$ calcium nitrate, it is assumed that the flocculating power of A is only one-fifth that of calcium nitrate. The photographs, Plate IV, Fig. 1, show the character of the results obtained in three series of these experiments. It was found to be important to work with equal quantities of standard material of fairly uniform fineness of grain; the relations are obscured if the amount of

material varies too widely, or if it contains many coarser particles, because of their tendency to drag finer ones down with them on settlement.

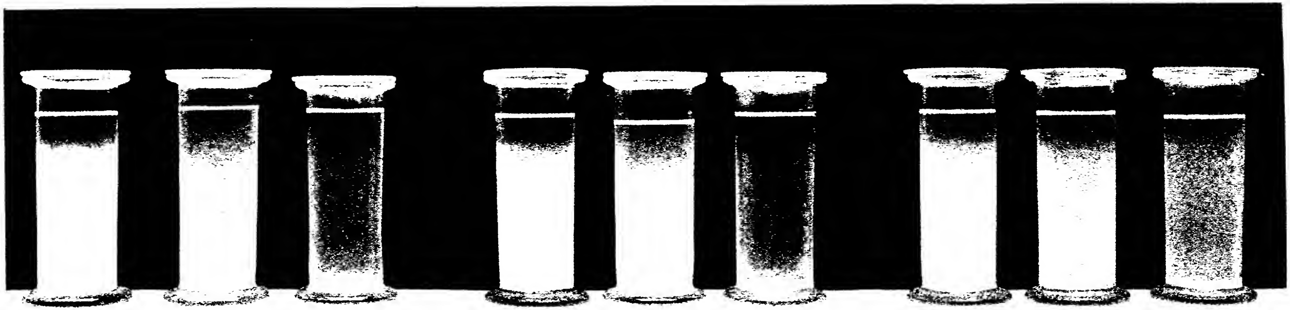
Only certain substances seem to be capable of indefinite suspension in water, of assuming what the authors call the 'natant' condition, however finely divided they may be. For example among the class of bodies likely to be found in soils, hydrated ferric oxide, obtained by grinding natural limonite or iron rust into a very fine state, flocculated spontaneously and settled down rapidly after shaking up with water. Yet the fineness of grain was such as would allow it to assume the natant condition, and indeed it did become so if a little ammonia were added to the water. Hydrated alumina, in the form of finely ground bauxite, behaved in a similar manner, and silica after long rubbing in an agate mortar did not yield wholly satisfactory results; even the finest precipitated silica makes a suspension that remains turbid for but a short time. In a truly 'natant' suspension the particles are just defined when enlarged one thousand diameters; they are then seen to be in rapid Brownian motion, but if a trace of flocculating salt be introduced at the edge of the cover-glass as it diffuses into the field of view the particles will be observed to lose their motion and draw together into little clots. These aggregates are however not permanent, but can be at once broken up so as to restore the 'natant' condition by washing away the flocculating salt.

This reversibility distinguishes true flocculation from cases of the clearing of an opalescent liquid by the growth of the suspended particles, such as is seen when a dilute silver solution is precipitated as chloride and the presence of an excess of acid induces the accretion of the very fine particles.

The following experiments illustrate the nature of the changes taking place during flocculation.

1. Up to a certain point, flocculation, as measured by the rapidity of settlement, is proportional to the amount of flocculating salt added. Beyond that limit all solutions flocculate alike.

Suspensions were made up in the usual way by mixing 200 c.c. of water with 10 c.c. of the strong kaolin suspension and a measured quantity of calcium nitrate solution to bring the strength of the liquid to the concentration specified in the second column.



n 2000 n 1000 n 500
Calcium Chloride

n 2000 n 1000 n 500
Calcium Nitrate

n 2000 n 1000 n 500
Calcium Sulphate

I. Comparative flocculating effects of Hydrochloric, Nitric, and Sulphuric Acids (as Calcium Salts).

1 2 3 4 5

II. Acetic Acid
1. n 40,000. 2. n 20,000.
3. n 10,000. 4. n 5000.
5. n 2500.

1 2 3 4 5

III. Comparison of acids and their sodium salts at n 10,000. In each pair the left hand jar contains acid, the right hand jar its sodium salt.
1. Hydrochloric. 2. Sulphuric. 3. Acetic. 4. Mono-chlor-acetic. 5. Tri-chlor-acetic.

EXPERIMENT I. *Calcium nitrate.*

No.	Strength of solution, fractions of normal	
1	100,000	After 90 hours, not clear but forming layers.
2	40,000	,, ,, clearing a little.
3	20,000	Not quite clear in 90 hours.
4	10,000	Clear in 48 hours.
5	4,000	Showed flocculation in 4 hours, clear in 9 hours.
6	2,000	,, ,, ,,
7	1,000	After 4 hours nearly clear.
8	400	,, ,,
9	200	,, ,,
10	100	,, ,,
11	40	,, ,,
12	20	,, ,,

In this case a concentration of $n/1000$ seems to be about the limit for the amount of kaolin used, increase of concentration beyond that point does not cause any quicker settlement. This experiment may be compared with a parallel series where sodium chloride replaced the calcium nitrate.

EXPERIMENT II. *Sodium chloride.*

No.	Strength of solution, fractions of normal	
1	100,000	No change after 70 hours.
2	40,000	
3	20,000	Clearing in layers after 70 hours.
4	10,000	,, ,, ,,
5	4,000	Clear after 48 hours.
6	2,000	Flocculating but not clear after 9 hours.
7	1,000	Clear after 9 hours.
8	400	After 4 hours nearly clear.
9	200	,, ,,
10	100	,, ,,

Here the limit may be taken as $n/400$ sodium chloride, as compared with $n/1000$ calcium nitrate, all the suspensions containing the two salts in these ratios pursuing a parallel course.

The results with calcium nitrate and sodium chloride were typical of those obtained with a number of other salts, the only variation being the limit of concentration above which complete flocculation set in. Of course some salts will not induce flocculation at all, a fact which will be considered later.

No evidence was obtained of reversal effects, such as a substance ceasing to flocculate when its concentration was increased above a certain strength, or of the existence of successive maximal and minimal points of flocculation as described by Joly (*loc. cit.*), though experiments were made with sodium nitrate up to concentrations of $2n$ or 17 per cent. of the salt.

2. The reaction between the flocculated substance and the flocculant is a quantitative one.

The following experiment will illustrate this point; the amount of kaolin in the suspensions was varied, being 1, 2, 4, and 8 times the normal respectively. Similarly the amount of the flocculating salt, calcium nitrate, was varied so as to obtain three series of jars containing the same ratio of kaolin to salt, the water being varied to make up the same bulk in each case.

EXPERIMENT III.

No.	c.c. of kaolin suspension	c.c. of $n/100$ calcium nitrate added	Ratio of kaolin to calcium nitrate
1	1	1	1
2	2	1	2
3	4	1	4
4	8	1	8
5	2	2	1
6	4	2	2
7	8	2	4
8	4	4	1
9	8	4	2
10	8	8	1

Under these conditions 1, 2, 3, 4 cleared in succession though they all contained the same concentration of calcium nitrate. Numbers 1, 5, 8 and 10, however, cleared approximately together, as afterwards did 2, 6, and 9, and still later 3 and 7. Suspensions containing the same ratio of kaolin to calcium nitrate cleared simultaneously, whatever the absolute amounts of the substances that were present in the liquid. In other words, a certain amount of salt is required to flocculate a given amount of kaolin, a conclusion which would also follow from the existence of a limit of concentration required to bring about flocculation in a given kaolin suspension, as described in the previous section. To carry out this experiment successfully it is of course necessary not to overpass this limit, otherwise all the suspensions will settle at the same rate. Again it is necessary to work at great dilutions and with small quantities of kaolin, because the relationship becomes obscured by the tendency of the higher concentrations of kaolin to clear more rapidly. When the flocculated kaolin is in any quantity it mats together, falls quickly, and to some extent clears the liquid behind it. In other words, a dense turbid liquid clears more effectually than a thin one, and this is true whether it is flocculated or not. But with care the quantitative relationship can be verified for various salts.

3. The process of flocculation does not appear to be accompanied by any removal of the salt from solution by the flocculated kaolin

(adsorption or laking out), nor by any selective absorption of base from the salt, so as to give rise to acidity in the liquid after flocculation had taken place. Picton and Linder (*loc. cit.*) found that the flocculation of arsenious sulphide by barium chloride was accompanied by a withdrawal of barium from solution, so that the resulting medium was acid. Working with kaolin no such acidity could be detected, and other experiments in the Rothamsted Laboratory (see Hall and Gimingham, *Trans. Chem. Soc.*, 91, 1907, 677) show that soluble salts such as cause flocculation merely interchange bases with the complex hydrated double silicates which make up kaolin.

Experiments were also made to ascertain if a change in conductivity accompanied the process of flocculation, as follows:—

A cylinder of Jena glass holding about 300 c.c. was immersed in a constant temperature bath with glass sides. A pair of platinum electrodes, held rigid by tubes (of Jena glass) containing the leads and suitable cross-pieces above and below, were completely immersed in the cylinder, which also contained a stirrer of silver. Usually the experiment began by measuring the conductivity of a measured volume of pure water in the cylinder, and determining the increase brought about by the addition of 10 c.c. of a solution of flocculating salt kept in the same bath. The required amount of kaolin suspension, also kept in the same bath, was then run in and the change in conductivity determined again. A previous blank experiment gave the conductivity of the kaolin suspension when diffused alone in the given volume of pure water.

Two of the experiments may be given, conductivities being expressed in reciprocal ohms per c.c.

EXPERIMENT IV. *Barium chloride* ($n/350$) *and kaolin* (0·2 *gram*).

The water used had a conductivity	$=2{\cdot}72\times10^{-6}$
On addition of the kaolin suspension it became	$=25{\cdot}35\times10^{-6}$
Increase due to the kaolin	$=22{\cdot}63\times10^{-6}$
On further addition of barium chloride the conductivity of the whole became	$=352{\cdot}8\times10^{-6}$
Increase due to the barium chloride	$=327{\cdot}45$
Starting the reverse way—	
Water	$=2{\cdot}99\times10^{-6}$
After the same amount of barium chloride had been added	$=331{\cdot}9\times10^{-6}$
Increase due to the barium chloride	$=328{\cdot}91\times10^{-6}$
On a further addition of kaolin	$=341{\cdot}5\times10^{-6}$
Increase due to the kaolin	$=9{\cdot}6\times10^{-6}$
Summarising—	
Increase of conductivity caused by barium chloride in water only ...	$=328{\cdot}91$
Increase of conductivity caused by barium chloride in kaolin suspension	$=327{\cdot}45$
Increase of conductivity caused by kaolin in water only	$=22{\cdot}63$
Increase of conductivity caused by kaolin in barium chloride solution	$=9{\cdot}6$

Thus in neither case did the flocculation result in lowering the conductivity of the salt solution, though when the kaolin was added to the barium chloride the increase in conductivity was not quite so great as would be expected from simply adding the conductivities of the separate constituents together.

EXPERIMENT V. *Aluminium sulphate* ($n/350$) *and kaolin* (0·2 *gram*).

Water alone	$=1{\cdot}35 \times 10^{-6}$
Water + aluminium sulphate	$=227{\cdot}6 \times 10^{-6}$
Water, aluminium sulphate, and kaolin	$=247{\cdot}5 \times 10^{-6}$
Conductivity added by the kaolin	$=19{\cdot}9 \times 10^{-6}$
Conductivity added by kaolin to pure water	$=22{\cdot}63 \times 10^{-6}$

EXPERIMENT VI. *Finely powdered natrolite* (0·2 *gram*) *and water.*

Water alone	$=2{\cdot}72 \times 10^{-6}$
Water and natrolite	$=6{\cdot}61 \times 10^{-6}$
Increase of conductivity due to natrolite alone	$=3{\cdot}89 \times 10^{-6}$

EXPERIMENT VII. *Natrolite* (0·2 *gram*) *and barium chloride* ($n/350$).

Barium chloride solution	$=336{\cdot}0 \times 10^{-6}$
After addition of natrolite	$=329{\cdot}3 \times 10^{-6}$

EXPERIMENT VIII. *Natrolite* (0·2 *gram*) *and ammonium chloride* ($n/350$).

Ammonium chloride solution	$=390{\cdot}8 \times 10^{-6}$
After addition of natrolite	$=381{\cdot}3 \times 10^{-6}$

In the two latter cases the addition and flocculation of natrolite were accompanied by a slight reduction in the conductivity of the solution, but this may be explained by the fact that barium or ammonium in the solution was to some extent replaced by sodium from the natrolite, *i.e.* by an element with a lower specific conductivity at those dilutions employed.

Other experiments were tried, but there was no evidence of any such drop in conductivity as would be occasioned by the removal from solution of any appreciable fraction of the salt.

4. The next stage in the experiments was to ascertain the comparative flocculating powers of a series of salts. The method of work was in all cases the same; varying amounts of the salt under investigation were added to the jars containing suspensions of kaolin made in the usual way, and these were matched against a standard series started at the same time with varying amounts of calcium nitrate as flocculant. It was early found that the flocculating power of the salts of a given metal is not independent of the acid, so that in

the comparisons of the various metals, chlorides had to be tested against chlorides, sulphates against sulphates, and so on.

The following list shows the comparative flocculating power of equi-normal solutions of the various metals tried, when the effect of the acid is eliminated; calcium nitrate being taken as a standard = 10.

H_2SO_4	20	HCl	> 20	HNO_3	> 20
$Al_2(SO_4)_3$	20				
$CaSO_4$	> 5	$CaCl_2$	> 10	$Ca(NO_3)_2$	10
$MgSO_4$	< 5			$Ba(NO_3)_2$	10
K_2SO_4	< 1	KCl	> 2	KNO_3	> 2
Na_2SO_4	0·5	NaCl	> 1	$NaNO_3$	< 1

The most powerful flocculators are therefore the free acids, though the aluminium salts come very close, possibly because they are so completely hydrolised in solution. The trivalent aluminium is more effective than the divalent elements calcium and magnesium, which in their turn flocculate better than sodium or potassium; but there is no evidence for the ratios 1 : 32 :: 1024 between mono-, di- and trivalent elements, which were found by Picton and Linder and justified on theoretical grounds by Whetham. Moreover the monovalent elements do not agree among themselves, potassium being about twice as effective as sodium, and hydrogen ten times greater still.

It should be remembered that the dilution of the solutions causing flocculation in these experiments is very great, usually about one-thousandth normal, so that the salts must be regarded as completely ionised.

It has already been stated that the flocculating power of a salt depends on the nature of the acid as well as of the base; a number of experiments were made to ascertain the relative order of the three acids—hydrochloric, nitric and sulphuric. The same relation was obtained whether the free acids or their salts were used, the best measurements of the ratios being

Hydrochloric acid	20
Nitric acid	19
Sulphuric acid	13

These ratios are not unlike the relative ionic dissociations of the three acids, as measured by their electrical conductivities, but any theory founded on such an agreement is not borne out by the behaviour of other acids like acetic. Acetic acid proved to be a very effective flocculant, being but little less active than sulphuric acid, 9 as against 13 on the above scale. Substitution of chlorine in the acetic acid made but

little difference, monochloracetic acid being rather more effective than the di- and tri-chloro acids, and all being a little better than acetic acid itself. The range of difference can hardly however be expressed in figures. On the other hand, amid-acetic acid did not flocculate at all.

Of the other acids tried oxalic and tartaric caused flocculation, and may be represented by 2·5 on the scale, oxalic being rather the more effective. Citric acid and phenol did not flocculate at all.

Perhaps the most interesting case is however afforded by the behaviour of the hydrates: it has always been known that the free alkaline hydrates are powerful deflocculators, a trace of ammonia, potash or soda will immediately cause clay particles to assume the 'natant' condition. This is also true of the carbonates and bi-carbonates of the alkalis and of similar salts of weak acids like the borates and the phosphates. Calcium hydrate is however used in practice to flocculate clay, as is shown by the improved tilth resulting from the application of quicklime to clay soils. In this case however the action is complicated by the presence of carbon dioxide, which will on the one hand precipitate the calcium as carbonate, dragging down at the same time the suspended material, and again will give rise to soluble calcium bi-carbonate.

Several determinations were made of the flocculating power of calcium hydrate, care being taken to deprive all the solutions used as far as possible of carbon dioxide and also to keep the suspensions out of contact of the air during their standing. Under these conditions calcium hydrate had a flocculating power of about 3, when calcium nitrate is rated at 10 for comparison; the concentration of the suspension in calcium hydrate being about one-thousandth normal. It will thus be seen that the flocculating effect of calcium hydrate is positive, whereas the hydrates of potassium, sodium, and ammonium give negative effects. Barium hydrate gave a very similar result to calcium hydrate.

It may perhaps be concluded that hydroxyl ions have a negative flocculating action, which is able to overpower the positive action of the potassium, sodium and ammonium ions, but is not equal to the greater positive action of the calcium or barium ions.

When calcium bi-carbonate was tried it proved a very effective flocculator, being a little better than equivalent solutions of calcium nitrate, though a numerical value could not be given to the superiority. Nor could this be attributed to the excess of carbon dioxide, for

measurements of a solution of free carbon dioxide only give the low value of 0·5 for carbon dioxide.

5. A few experiments were tried with other materials than kaolin. Bauxite and limonite respectively were ground fine in an agate mortar, and further reduced by wet grinding in an improvised ball-mill for a day or two. Precipitated silica was also prepared and used after igniting and grinding. Neither bauxite nor limonite would assume the 'natant' condition in pure water; they flocculated and settled immediately. The presence of alkali in concentrations of $n/2000$ to $n/500$ however caused them to remain suspended; sulphuric acid flocculated, but aluminium sulphate at the same concentrations caused the bauxite to remain suspended. In a second series the bauxite with aluminium sulphate again held up better than the check with pure water, but magnesium sulphate tried at the same time permitted of flocculation like the pure water. A small sample of allophane, $Al_2(HO)_2SiO_4 + xH_2O$, was scraped off and reduced to a fine powder; this also would not assume the natant condition in pure water, though it would remain suspended when a trace of free alkali was added.

The experiments with these bodies were abandoned because of the impossibility of obtaining true suspensions in water alone.

With ignited silica there was no difference between the suspensions in pure water and those containing either acid or alkali; flocculation could be brought about by aluminium sulphate, but it was difficult to make satisfactory suspensions.

The authors have failed to find any satisfactory theory that embraces the whole of the observed facts.

The electrical theory developed by Whetham, depending upon the discharge of the suspended particles by the free ions, would seem to fail, because

(1) The flocculation depends upon the ratio of amounts of flocculating material and flocculant, and not merely on the concentration of the flocculant.

(2) The different acids and the salts of different acids show great variations in their flocculating power, nor are these variations dependent on the degree to which ionisation takes place. Acetic acid and the acetates for example have much the same value as sulphuric acid and the sulphates, and chlorinating the acetic acid has little effect on the result.

(3) The ratio between the flocculating power of mono-, di- and trivalent metals is far from that expected by the theory.

(4) No change takes place in the conductivity of the solution during and after flocculation.

The electrical theory sketched by Joly is similarly not justified by the facts.

Again flocculation cannot depend upon the hydrolysis of the salts employed and the action of the free acid, except perhaps in the case of the aluminium salts. Hydrolysis alone would not explain the effectiveness of such salts as calcium chloride which are but slightly hydrolised, nor the high position of the acetates, nor the fact that the chloracetates possess much the same value as the acetates.

The authors also consider that the conductivity experiments and the quantitative measurements with stronger solutions already referred to must dismiss any theory founded on the selective precipitation of the base of the flocculant on to the suspended particles and a resulting 'laking out.'

Nor can any theory be built up on an hypothesis of preliminary chemical action between the salt and the particles (though the salts in question do act upon kaolin), accompanied by aggregation. Such an explanation is sufficiently negatived by the fact that the action is reversible; the flocculated material can be suspended afresh in pure water when the salt has been washed away.

It was thought that the preliminary chemical action might induce some change in the surface tension existing between the solid particles and the solution; this however is not a sufficient hypothesis as the following experiment shows. Kaolin was flocculated by 1/10,000 normal ammonium chloride solution, washed free from salt, and re-suspended in pure water. On adding ammonium chloride to again bring up the solution to 1/10,000 normal the kaolin was flocculated afresh. No new chemical action could have taken place, because on the first occasion the kaolin would have completed its reaction with the ammonium chloride of that concentration so that no further change would take place on a second contact with another solution of the same strength.

Before any theory of flocculation can be reached it is probably necessary to determine the conditions which must be satisfied before a given substance will assume the 'natant' state. While size is of course a factor, for particles above about 0·004 mm. will not remain suspended for any length of time, chemical composition also comes in to determine whether the particles below this limit will possess a Brownian motion and remain apart instead of aggregating when

suspended in water. Of the substances examined, only kaolin natrolite and the various clays, which may be regarded as impure kaolins, would become natant; hydrated alumina, hydrated ferric oxide, silica, and even allophane, another hydrated silicate of alumina, fall rapidly and completely from suspension to form a matted deposit at the bottom of the vessel. Furthermore it can be shown that it is not the zeolitic character of the kaolin &c. that confers the natant character. A sample of kaolin was ignited for some time at a low red heat, more than sufficient to destroy any zeolitic compounds; this sample was then graded by suspension in water like the original kaolin and a fraction separated that would remain suspended for 24 hours. A number of suspensions were then made up containing equal dilutions of ignited and unignited kaolin respectively, and various amounts of calcium nitrate added to induce flocculation; the ignited kaolin assumed a true 'natant' condition, but was more readily flocculated than the unignited kaolin. The only feature that seems to distinguish the natant from the non-natant bodies, is that the former—kaolin and natrolite—are essentially double salts of an alkaline metal which give rise to a trace of free soluble alkali when they are mixed with water. Both kaolin and natrolite contain alkaline metals, and fresh mineral specimens when ground in an agate mortar with pure water will gradually blue a strip of red litmus paper immersed in the thin paste when it is allowed to dry spontaneously. On the other hand the specimen of allophane used seemed to be free from the alkali metals, as also are limonite, bauxite and the silica used; these latter substances would only become natant when free alkaline hydrates were added to the suspensions.

If then the natant condition is dependent on the presence of traces of free alkali derived from the partial hydrolysis of the suspended material, it is clear that the free acids should be the best flocculators; it is also reasonable to suppose that the presence of a salt, which would react in the way of double decomposition with the part of the material that would be hydrolised by pure water, would also tend to suppress any free alkaline hydrates. Again the general quantitative relationship between suspended material and flocculant would also follow; even the departure from this rule in that a concentrated suspension requires a smaller proportion of flocculant than a dilute one, can be explained by assuming that the amount of hydrolysis of the natant material would be greater when it was in contact with a larger bulk of water.

Beyond this point, that the natant condition is dependent upon the presence of free alkaline hydrates and that flocculation ensues when these are neutralised or driven back into combination with the suspended solid, the authors prefer to attempt to put forward no further theory, but merely to record a series of observations and measurements that are critical of the theories already advanced, in the hope that they may afford material to induce some physicist to take up the question afresh. It would appear to be necessary first to arrive at an explanation of Brownian motion.

THE EFFECT OF FUNGICIDES UPON THE ASSIMILATION OF CARBON DIOXIDE BY GREEN LEAVES.

By ARTHUR AMOS, B.A.

In some of the earlier experiments in which Bordeaux Mixture was used as a fungicide, it has happened that neither the part sprayed nor the control has suffered from disease at all; yet it has been recorded that the sprayed part has continued green for a longer period and has produced a larger crop.

Again "Flowers of Sulphur" is commonly blown on to the hop plant by hop growers in Kent shortly before picking, the object being to cause the hops to retain their green colour for a longer period.

It might be supposed therefore that both Bordeaux Mixture and Flowers of Sulphur, when applied to a green leaf, may increase the amount of carbon dioxide assimilation independently of any possible fungicidal action.

Frank and Krüger (*D. Landw. Gesell.* 1894, 2) state that both assimilation and transpiration are increased in leaves treated with Bordeaux mixture, owing to the stimulating action of the copper salts.

On the other hand more recent experiments by Ewert (*Landw. Jahr.* XXXIV. (1905), 233) and others have shown that plants sprayed with Bordeaux Mixture gave a diminished crop, although they retained their green colour for a longer period than the unsprayed.

The following experiments were devised to ascertain whether the assimilation of carbon dioxide was affected in any way by the application of Bordeaux Mixture or Flowers of Sulphur, and whether any stimulus effect could be demonstrated.

Method employed.

In each experiment a pair of leaves on the same plant were selected; where possible, opposite leaves on the same stem; the pair were selected as nearly as possible identical in age, shape, and size.

The leaves remained attached to the growing plant throughout the experiment; one being used as a control to the other.

In order to determine the assimilatory powers of the leaves the method employed by Brown and Escombe (*Proc. Royal Soc.* B LXXVI. (1905), 30) was used; the leaves were placed separately into glazed cases, in which they were exposed to sunlight, and through which air was drawn; the volume of air and content of CO_2 in the issuing air were then determined, and knowing the original CO_2-content of air, the amount of CO_2 assimilated by each leaf could be calculated.

By this means the ratio between the assimilatory powers of the two leaves was obtained; this was twice repeated to ensure accuracy, and then one of the leaves was treated with the fungicide.

The ratio between the assimilatory powers of the two leaves was again determined on three occasions after the application of the fungicide, when any alteration in the ratio may be set down to the application of the fungicide.

The apparatus was that originally used by Brown and Escombe and was kindly lent by Dr Horace Brown; it will be sufficient here to state that the leaves under experiment were simultaneously exposed to the light in their respective cases and that the air-current was drawn over each at as nearly as possible the same rate. For the details of the corrections that have to be made for temperature and pressure, and the titration of the alkaline absorbing solution, Brown and Escombe's paper should be consulted. The absolute content of carbon dioxide in the air at the time of each experiment was not determined.

Calculation of Results.

Brown and Escombe[1] have shown that the CO_2-content of the air at the Jodrell laboratory at Kew varies on rare occasions, and only during thick fog, between the limits of 2·8 and 3·2 parts per 10,000; with a mean to 2·9 parts per 10,000 in 91 experiments.

During the experiments the CO_2-content of the air was occasionally determined, and this always fell well within the above-mentioned limits.

This mean amount, 2·9 parts per 10,000, was assumed to be the CO_2-content of the air entering the leaf-cases; and since it was not the absolute CO_2-assimilation, but merely the relative assimilation between

[1] *Proc. Roy. Soc.* Vol. B LXXVI. 1905, p. 113.

two leaves that was required, this assumption can only cause an insignificant error.

On this assumption the amount of CO_2 absorbed per sq. decimeter of leaf area per hour was then calculated; let it be x cubic centimeters.

Now the mean CO_2-content of the air in contact with the leaf in the case is not the CO_2-content of the outside air, but the mean between the CO_2-contents of the entering and issuing air; this mean value is calculated, let it be y parts per 10,000; x then represents the amount of CO_2 absorbed per sq. dcm. of leaf area per hour from air containing y parts CO_2 per 10,000.

Brown and Escombe[1] have shown that the rate of assimilation of CO_2 by green leaves is proportional to the CO_2-content of the air, when the CO_2-content of the air is of the same order as 2·9 parts per 10,000.

In order to make the results comparable they are all calculated to show the amount of CO_2, which would have been assimilated from air containing 2·9 parts per 10,000; *e.g.* in the above case, the leaf would have assimilated $x \times \frac{2{\cdot}9}{y}$ c.c. CO_2 per sq. dcm. per hr., if the mean CO_2-content of the air surrounding the leaf was 2·9 parts per 10,000.

In this way the assimilatory powers of the two leaves under experiment were calculated, and the ratio between these numbers gives a ratio between the assimilatory efficiency of the two leaves.

SERIES I.

After a few preliminary trials to see that the whole apparatus was in working order, the real experiment was begun on June 27th.

In this case the effect of Bordeaux Mixture was tried upon the leaves of the hop.

Early in the spring a hop-root was planted just outside the laboratory window, and the shoots from this were trained upon strings so that, when required, the stems and leaves could be brought into any desired position without injury to the plant.

On June 27th one of the stems was placed upon a bench outside the laboratory window; two opposite leaves were selected, and put into two leaf cases placed side by side upon the bench, and the moveable glass plates cemented in position in each case with soft wax.

[1] *Proc. Roy. Soc.* Vol. LXX. 1902, p. 397.

As soon as the glass plates were fixed in position, a steady stream of air, which passed through rubber tubes from the leaf cases outside to the absorption apparatus within the laboratory, was started, and continued for six hours or more, direct sun being screened from the leaves by a thin linen sheet stretched above.

At the end of this time the amounts of CO_2 absorbed by the caustic soda in the Reisets[1] were estimated, and the absorption of CO_2 by the leaves calculated as previously explained.

On July 3rd and 4th the absorption of CO_2 by the two leaves was again determined under normal conditions.

Then, after the trial on July 4th, Bordeaux Mixture was applied to leaf *B* by gently moistening both the upper and lower surfaces with a sponge soaked in this fungicide.

On July 5th, 6th, and 7th the absorption of CO_2 by the two leaves was determined, and again after an interval of about three weeks on July 26th and 27th.

Table I. contains the results of these experiments; in the first column is the date upon which the experiment was made; the second column gives the volumes of CO_2 absorbed per square decimeter of leaf surface per hour by leaf *A*, the control leaf; the third column gives the volumes of CO_2 absorbed per square decimeter of leaf surface per hour by leaf *B*, which was treated with Bordeaux Mixture on July 4th; the fourth column gives the duration of each experiment; and the fifth column gives the ratio between the figures in columns 2 and 3, and expresses a ratio between the assimilatory powers of the two leaves.

Dealing in the first place with the experiments on June 27th, July 3rd and 4th, it will be noticed that the figure, indicating the volume of CO_2 absorbed by each leaf, varied from day to day; this is due to two factors, firstly to the size of the stomatal openings, which in turn are dependent on a variety of causes, and secondly to the CO_2-content of the air.

Since, however, these factors affect the assimilation of each leaf to the same extent, the ratio between the amounts of CO_2 assimilated by each leaf is not affected by them; and in the last column of the table it is seen that this ratio is fairly constant, varying from 1 : 1·228 to 1 : 1·141, and giving a mean ratio of 1 : 1·178.

One would naturally suppose that this ratio would approximate to 1 : 1 and the fact that in this case it does not do so can only be explained by supposing that leaf *B* was originally more efficient than leaf *A*.

[1] See Amos. This *Journal*, vol. I. 322.

Coming now to the second part of the table, in which leaf *B* ha been coppered, it is seen at once that the assimilation of the coppered leaf *B* had diminished in comparison with that of the untreated leaf *A*, and that the mean of the ratios between the amounts of CO_2 assimilated by leaf *A* to *B* had fallen from 1 : 1·178 (ratio when both leaves untreated) to 1 : 1·047 (ratio when leaf *B* is coppered).

The application of the Bordeaux Mixture had thus reduced assimilation.

TABLE I.

Effect of Bordeaux Mixture on the assimilation of the Hop-leaf.

Date	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *A*	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *B*	Duration of experiment hrs.	min.	Ratio of CO_2 absorbed per sq. dcm. per hour by *A* to *B*
June 27th	2·42	2·97	6	0	1 : 1·228
July 3rd	2·22	2·59	6	0	1 : 1·165
July 4th	2·06	2·35	6	30	1 : 1·141
					Mean 1 : 1·178
	On July 4th leaf *B* was coppered				
July 5th	3·04	3·14	8	0	1 : 1·035
July 6th	2·79	3·14	8	0	1 : 1·125
July 7th	2·37	2·33	8	30	1 : ·980
					Mean 1 : 1·047
	After an interval of 3 weeks.				
July 26th	1·22	1·46	6	0	1 : 1·198
July 27th	1·58	1·88	6	15	1 : 1·190
					Mean 1 : 1·194

Area of leaf $A = 1·262$ sq. dcm. Area of leaf $B = 1·260$ sq. dcm.

It was thought [because of the statements that plants which have been sprayed with Bordeaux Mixture remain green longer than untreated plants] that perhaps, at a still later stage, the coppered leaf *B* would surpass its control leaf *A* in assimilation.

The results of the experiments on July 26th and 27th (when both leaves were beginning to show signs of age) indicate that the assimilatory ratio had returned to about its original value: thus showing that the deleterious effects of the application of Bordeaux Mixture had passed away.

It must however be noticed that the assimilatory powers of both leaves had then dropped to nearly half their original value.

The leaves, shortly after this, began to turn brown, and the sprayed leaf did not keep green longer than the untreated one.

Series II.

Bordeaux Mixture on the Vine.

In this series the leaves of a grape-vine were used; the vine was planted in a large flower-pot, and the plant consisted of a central axis about one foot long, growing from which were five young branches about four feet long at the time of the experiment.

The vine was placed in an unheated green-house in the spring, and the leaves were thus well developed and not bruised.

When required, the vine and pot were arranged outside the laboratory window, so that the stems and leaves could be placed horizontally on the bench.

The vine has alternate leaves, so another device was employed to obtain leaves of equal size and age. Two of the branches from the main axis grew very uniformly and the 7th leaf on each began to unfold about the same time; these leaves, when developed, appeared to the eye equal in all respects, and were selected for these experiments; the assimilatory powers of these two leaves were determined as in Series I. three times before the application of the Bordeaux Mixture.

The Bordeaux Mixture was applied on Aug. 1st in this case by means of a tiny syringe, since it was thought that the application by means of the sponge might damage the leaf.

The assimilatory powers were again determined three times, and then after an interval of 10 days twice more.

Table II. shows the results of this series of experiments, and is arranged exactly as Table I.; the results in this case show still more clearly the effect of the Bordeaux Mixture in diminishing assimilation.

The table shows that, before the application of the Bordeaux Mixture, the assimilatory powers of the two leaves were almost identical, the ratio between them being 1 : 1·02.

After the application of the Bordeaux Mixture to leaf *B*, the assimilatory power of *B* compared with the control *A* was depressed by about $\frac{1}{5}$, the ratio now being 1 : ·79.

TABLE II.

Effect of Bordeaux Mixture on the assimilation of the Vine-leaf.

Date	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *A*	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *B*	Duration of experiment hrs.	min.		Ratio of CO_2 absorbed per sq. dcm. per hour by *A* to *B*
July 28th	2·28	2·47	8	25		1 : 1·08
July 29th	1·87	1·86	7	30		1 : ·99
July 31st	1·86	1·84	6	0		1 : ·99
					Mean	1 : 1·02
	On Aug. 1st leaf *B* was coppered.					
Aug. 4th	1·56	1·17	7	0		1 : ·75
Aug. 5th	1·72	1·43	4	45		1 : ·83
Aug. 10th	1·97	1·57	5	45		1 : ·79
					Mean	1 : ·79
Aug. 18th	2·17	2·08	6	50		1 : 1·04
Aug. 20th	1·78	1·75	5	0		1 : 1·02
					Mean	1 : 1·03

Area of leaf $A = 1·395$ sq. decimeters. Area of leaf $B = 1·335$ sq. decimeters.

It is again noticeable that this depressing effect of the Bordeaux Mixture passes off after a time, and on Aug. 18th and 20th the assimilatory power of leaf *B* had risen to its original value, the ratio between *A* and *B* now being 1 : 1·03.

During this experiment the leaves grew somewhat and were still quite vigorous and green at the end; it would have been interesting to have continued this experiment longer, but this was unfortunately impossible.

SERIES III.

Bordeaux Mixture on the Jerusalem Artichoke.

In the third series the leaves of an artichoke were employed; artichoke plants, growing in the open ground, were transplanted in June into large flower-pots; at the time of the experiment they were about five feet high and growing vigorously.

During the experiments the plants were propped up on the bench as in the case of the vine.

The leaves of the artichoke are spirally arranged, but grow very thickly on the stem, so that it was quite easy to obtain two leaves on opposite sides of the stem, which were approximately equal in size and age.

Two such leaves were selected, and the assimilatory powers of each determined as in the previous series, firstly, when both were normal, secondly, when one leaf was coppered, and thirdly, a week after the application of the Bordeaux Mixture.

Table III. shows the same general results as the two preceding ones; the initial assimilatory power of the two leaves was nearly equal; after the application of Bordeaux Mixture to leaf *B* the assimilation of this leaf compared with the control leaf *A* was considerably diminished; the ratio between the assimilatory powers of *A* and *B* being 1 : ·84.

On Aug. 21st, eight days after the spraying, the amounts of CO_2 assimilated per sq. dcm. per hour had fallen considerably; this was due to the fact that the leaves, which in the case of the artichoke are comparatively short-lived, were beginning to show signs of age; further, as in the case of Series I. and II., the effect of the Bordeaux Mixture had diminished, and the assimilatory power of *B* compared to *A* had nearly regained its former value; the ratio between *A* and *B* being now 1 : ·93.

TABLE III.

Effect of Bordeaux Mixture on the assimilation of the Jerusalem Artichoke leaf.

Date	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *A*	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *B*	Duration of experiment hrs. min.	Ratio of CO_2 absorbed per sq. dcm. per hour by *A* to *B*
Aug. 9th	2·26	2·10	6 50	1 : ·93
Aug. 11th	2·84	2·97	5 30	1 : 1·05
Aug. 12th	2·50	2·42	5 0	1 : ·96
				Mean 1 : ·98
On Aug. 13th leaf *B* was coppered.				
Aug. 14th	2·57	2·08	5 30	1 : ·81
Aug. 15th	2·53	2·22	6 30	1 : ·87
				Mean 1 : ·84
Aug. 21st	1·69	1·58	5 0	1 : ·93

Area of leaf $A = 2·221$ sq. decimeters. Area of leaf $B = 2·241$ sq. decimeters.

SERIES IV.

Flowers of Sulphur on the Jerusalem Artichoke.

In this series the same procedure was followed as before; after the assimilatory power of the two leaves had been determined on two days, leaf *B* was dusted on both surfaces with flowers of sulphur, and then the assimilatory power of the two leaves was again determined.

It was found that the sulphur produced no effect upon the assimilatory power of the leaf; the mean of the ratios of the assimilatory powers before the application being 1 : ·94 and after 1 : ·96.

TABLE IV.

Effect of Sulphur on the assimilation of the Jerusalem Artichoke leaf.

Date	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *A*	No. of c.c. CO_2 absorbed per sq. dcm. leaf surface per hour by leaf *B*	Duration of experiment hrs. min.	Ratio of CO_2 absorbed per sq. dcm. per hour by *A* to *B*
Aug. 16th	2·50	2·42	6 0	1 : ·96
Aug. 17th	2·42	2·23	5 30	1 : ·92
				Mean 1 : ·94
	On Aug. 19th leaf *B* was dusted with sulphur.			
Aug. 21st	2·35	2·29	4 0	1 : ·98
Aug. 22nd	2·91	2·67	5 30	1 : ·92
				Mean 1 : ·96

Area of leaf *A* = 1·580 sq. decimeters. Area of leaf *B* = 1·530 sq. decimeters.

Appended is a table showing the relative assimilatory powers of leaves used by Brown and Escombe and others used by the author.

In both cases the assimilatory power has been calculated for air containing 2·9 parts per 10,000.

TABLE V.

Date and Experimenter		Plant used	CO_2 absorbed per sq. dcm. of leaf per hr.
25/8/98	Brown and Escombe	Helianthus annuus	4·07
4/9/00	,, ,,	Tropæolum majus	1·70
31/8/99	,, ,,	Catalpa bignonioides	5·28
26/6/00	,, ,,	Petasites albus	2·36
3/7/00	,, ,,	Polygonum Weyrichii	2·35
27/6/05	Amos	Humulus lupulus	2·42 and 2·97
28/6/05	,,	Vitis vinifera	2·28 and 2·47
9/8/05	,,	Helianthus tuberosus	2·26 and 2·10

Conclusions.

The results of the experiments show that the application of Bordeaux Mixture to the leaves of a plant diminishes the assimilation of CO_2 by these leaves for a time; after a time this effect passes off both in cases where the leaves begin to age as in Series I. and III. and also whilst the leaves still keep vigorous as in Series II.

It seems probable that the stomata are partially blocked up by the Bordeaux Mixture, and that consequently less air diffuses into the intercellular spaces of the leaf and less CO_2 therefore comes in contact with the absorption surface.

This is supported by the observations of Schander[1], who found that the application of Bordeaux Mixture diminished transpiration.

The experiments were carried out at the Laboratory of the Lawes Agricultural Trust at Rothamsted, to whom my best thanks are due.

I am also very deeply indebted to Dr Horace Brown, F.R.S., and to Mr A. D. Hall, M.A., for much advice and assistance, and to the former also for the use of his apparatus.

[1] *Land. Jahr.* xxxiii. 1904, 517.

THE CHEMISTRY OF STRENGTH OF WHEAT FLOUR.

By T. B. WOOD, M.A.

Drapers Professor of Agriculture in the University of Cambridge.

Part II. The Shape of the Loaf.

Before proceeding to discuss the immediate subject of this communication, it may be well to recapitulate shortly the views on the general question of the meaning of strength of flours which I expressed in the last number of this journal. It may perhaps be remembered that I suggested that strength, as defined by Humphries and Biffen, to be the capacity of making large well-piled loaves, must be a complex of at least two factors. One of these factors, that which regulates the size of the loaf, I showed to be the power of continuing to evolve carbon dioxide gas throughout the later stages of dough fermentation. I described how to measure this property by incubating in a bottle at 40° C. a mixture of 20 gms. flour, 20 c.c. water, and ½ gm. yeast, the bottle being connected to a gas measuring apparatus, and the volume of gas given off being read from time to time. A number of experimental figures were given, which showed that the size of the loaf which a flour could make, was directly connected with the rate at which it gave off gas in the later stages of dough fermentation, at the time when it was ready for the oven.

The other factor of strength, that which decides the shape of the loaf, I tentatively ascribed to the soluble salts present in the flour. Since then I have been able to work out this line much more fully, and my results are sufficiently advanced to enable me to suggest what appears to be a possible, or perhaps I may even say, a probable, explanation of this factor.

For the sake of clearness it may be well to again recapitulate. Recent work has shown that neither total nitrogen, nor total gliadin,

nor ratio of gliadin nitrogen to total nitrogen, can be taken as a satisfactory index of the baking value of a flour. It seemed to me therefore that only two possibilities remained. The undoubted difference between the properties of the gluten of strong and weak flours might be due, either to some fundamental difference in their chemical composition more deep-seated than that shown by the proportion of the gluten dissolved by alcohol of certain strength, which is the essence of gliadin determinations, or on the other hand to purely physical causes. I therefore prepared samples of gliadin, as described by Osborne and Harris[1], from a number of flours differing widely in baking properties, and submitted them to hydrolysis, by boiling for eight hours with concentrated hydrochloric acid. The percentage of amide nitrogen in each was then determined by distilling with magnesia, and titrating the ammonia in the distillate. The percentage of amide nitrogen in the samples of gliadin was found to be the same for flours of all strengths from 95 to 40. On this evidence I concluded that the gliadins of strong and weak flours were identical. The identity of the glutenin of the same flours was then established in a similar manner, and the next step was to turn to the physical properties.

Much light has recently been thrown on the physical properties of colloids, and it has been shown among other things that the physical properties of such substances depend to a great extent on their surroundings, that in fact it is almost impossible to define their properties without defining the conditions under which they exist. The work of Hardy[2] and that of Picton and Linder[3] is particularly interesting in this connexion, since it shows how the state of aggregation of a colloid is modified by the concentration of acid, alkali, or salt, in the solution with which it is in contact.

It seemed therefore worth while to determine the acidity, and the amount of soluble salts, in a number of flours of widely different baking properties. The results of some of these measurements were given in my last paper, already referred to, and it was pointed out how widely they varied, and how difficult it was to correlate their variations with baking properties. Thus the highest and lowest acidities were found in flours having the same bakers' marks, and there was no apparent connexion between the percentage of soluble ash and the bakers' marks, though there appeared to be some relation between bakers' marks and the ratio of soluble ash to total nitrogen. A second

[1] *J. Amer. Chem. Soc.* 1903, 323 and *Proteids of the Wheat Kernel.* Pub. Carnegie Inst. Washington, No. 84.

[2] *Proc. Roy. Soc.* LXVI. 95 and 110. [3] *Trans. Chem. Soc.* 1892, 148 and 1895, 63.

series of determinations did not make these relations more apparent, and it was at this stage of the investigation that Mr W. B. Hardy suggested that I should try to find some connexion between the properties of gluten and the composition of its surroundings by observing how its properties changed when it was immersed in solutions of varying concentration.

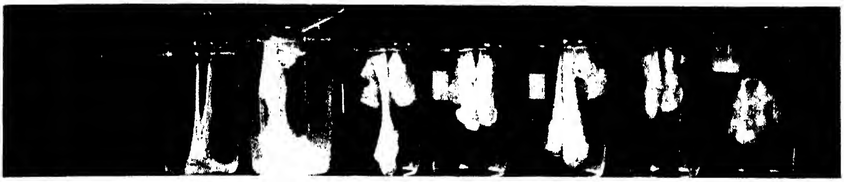
Accordingly a quantity of gluten was prepared from ordinary household flour, and small bits of it were immersed in solutions of which at first the acidity only was varied, the acid used being hydrochloric. After many trials the following method of experimenting was devised, and found to answer extremely well. A large number of small beakers were each marked at 80 c.c. Normal hydrochloric acid was then run in from a burette in sufficient quantity to make the required strength when diluted to the 80 c.c. mark. A small string of gluten about two inches long and as thick as a pencil was then pulled off the large lump and hung by its middle point over a V-shaped glass rod, the tips of the V resting on the edge of the beaker, and the gluten being immersed in the solution. When a mineral acid was used no other antiseptic was necessary. In all other cases the solutions were made up with water which had been shaken with toluene, and the beakers covered if possible to prevent the toluene from evaporating. Such bits of gluten require from 36 to 48 hours to attain equilibrium with the solution in which they are immersed.

Experimenting in this way it was found that gluten immersed in distilled water would retain its coherence for some time, in fact until what were probably bacterial changes intervened or, if the water were repeatedly changed, until all acids and salts had been removed. In very dilute hydrochloric acid however, *e.g.* N/1000, the gluten soon began to disintegrate and lose its coherence, and this change was more rapid as the concentration of the acid was increased up to about N/30. Further increase in concentration slowed down the rate of disintegration, until, at a concentration of N/12, the gluten again became permanently coherent, and much harder and more elastic, and less sticky, than in its original condition. Similar experiments were made with sulphuric, phosphoric, oxalic, acetic, lactic, citric, and tartaric acids. The behaviour of sulphuric, phosphoric, and oxalic acids differed only in degree from that of hydrochloric. Very dilute solutions of each caused the gluten to disintegrate, and strong solutions again produced coherence. The limiting concentration required to cause permanent coherence was, for sulphuric acid N/25, for phosphoric acid 1·75N, for

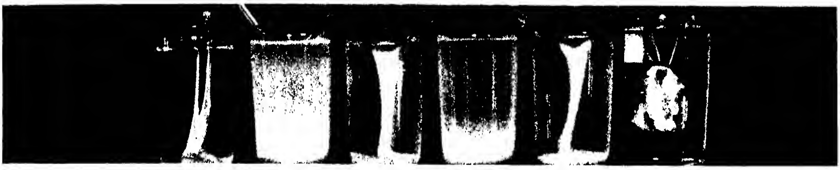
oxalic N/4. The other acids behaved entirely differently. Dilute solutions produced disintegration, and this became continuously more rapid as the solution was made more concentrated. No practicable concentration could be found above which coherence reappeared.

Flour however contains soluble salts as well as acids, and the next step therefore was to try the effect of immersing gluten in dilute solutions of the various acids containing varying proportions of different salts. The first pair tried were hydrochloric acid and sodium chloride, and the experiments were carried out as follows. A number of small beakers were prepared as before. Enough standard hydrochloric acid was run into each to make N/50 solution when the beaker was filled up to the mark. Standard solution of sodium chloride was then added in varying quantity, so as to make say N/1000, N/100, N/10, and the beakers were then filled up to the mark. The bits of gluten, prepared as before, were then immersed in the solution and left for 48 hours. At the end of this time the gluten which was immersed in N/50 acid and N/10 salt solution was firm and coherent, the other two had disintegrated. A second series was then prepared containing N/50 acid and salt solution of varying concentrations between N/100 and N/10. Proceeding in this way it was found that gluten in contact with N/50 hydrochloric acid required the presence of salt of the concentration of about N/12 in order to make it cohere. Further experiments showed that if the acid were either more or less concentrated than N/50, less salt was required to keep the gluten in the coherent condition. A number of measurements of the concentration of salt required to make gluten preserve its coherence in acids of several concentrations between N/1000 and N/12 were made, and the results are plotted in the curve for NaCl in Pl. VI. Fig. 1. In this and all the accompanying curves in Pl. VI. the ordinates represent the concentration of salt in gram equivalents per 1000 litres, the abscissae the concentration of acid on the same scale. The method of working and the appearance of the gluten in the different states are well shown in Pl. V.

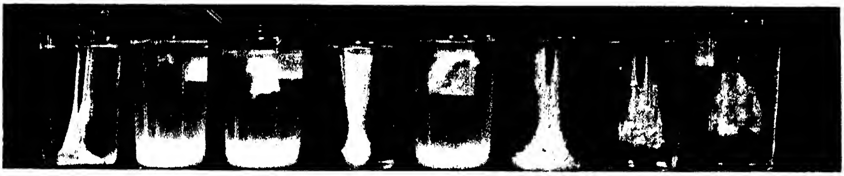
Pl. VI. Fig. 1 also shows similar curves for other salts beside sodium chloride. It is noticeable that the curves for the different salts are all of the same type, the chief differences being stated below. Three sodium salts were tried, chloride, sulphate, and phosphate. Of these chloride and phosphate are approximately equal in power of producing coherence, but chloride is more active in combination with more concentrated acid, phosphate with less concentrated acid. Sulphate is much more active than either chloride or phosphate, the ratio



HCl	0	10	10	10	20	20	100
NaCl	0	60	75	90	75	90	0



HCl	0	10	10	20	20	100
Na_2HPO_4	0	18	24	50	70	0



HCl	0	10	10	10	20	20	20	100
Na_2SO_4	0	20	25	40	20	40	60	0

HCl	10	10	10	20	20	40	40	40
$MgSO_4$	10	15	20	20	40	10	15	20

HCl	10	10	20	20	60
$Al_2(SO_4)_3$	8	12	10	20	5

The figures give concentrations in gram-equivalents per 1000 litres.

of their activities being approximately as follows:—chloride or phosphate : sulphate as 2 : 3. Sulphate resembles chloride in being more active with higher concentrations of acid.

The other three curves in Pl. VI. Fig. 1 show the results of similar experiments with the sulphates of sodium, magnesium, and aluminium. Evidently the activity is related in some way to valency, increasing as the valency increases. The ratio of the activity of sodium : magnesium : aluminium is roughly 1 : 2 : 4.

Similar results were obtained by experimenting with other acids. Pl. VI. Fig. 2 gives the curve for sulphuric acid and sodium chloride, Pl. VI. Fig. 3 that for phosphoric acid and sodium chloride, and Fig. 4 that for oxalic acid and sodium chloride. These curves are all of the same type as that for hydrochloric acid and sodium chloride. They differ only in the limits of concentration of acid required to keep the gluten coherent in the absence of salts.

Curves of quite another type are shown in Pl. VI. Fig. 5, the curve for lactic acid and sodium chloride. In this curve the behaviour of lactic acid is well shown. Gluten disintegrates in all strengths of this acid, the rate of disintegration being more rapid the more concentrated the solution. As the concentration of the acid is increased more and more sodium chloride must be added to preserve coherence. In this respect tartaric and citric acids resemble lactic.

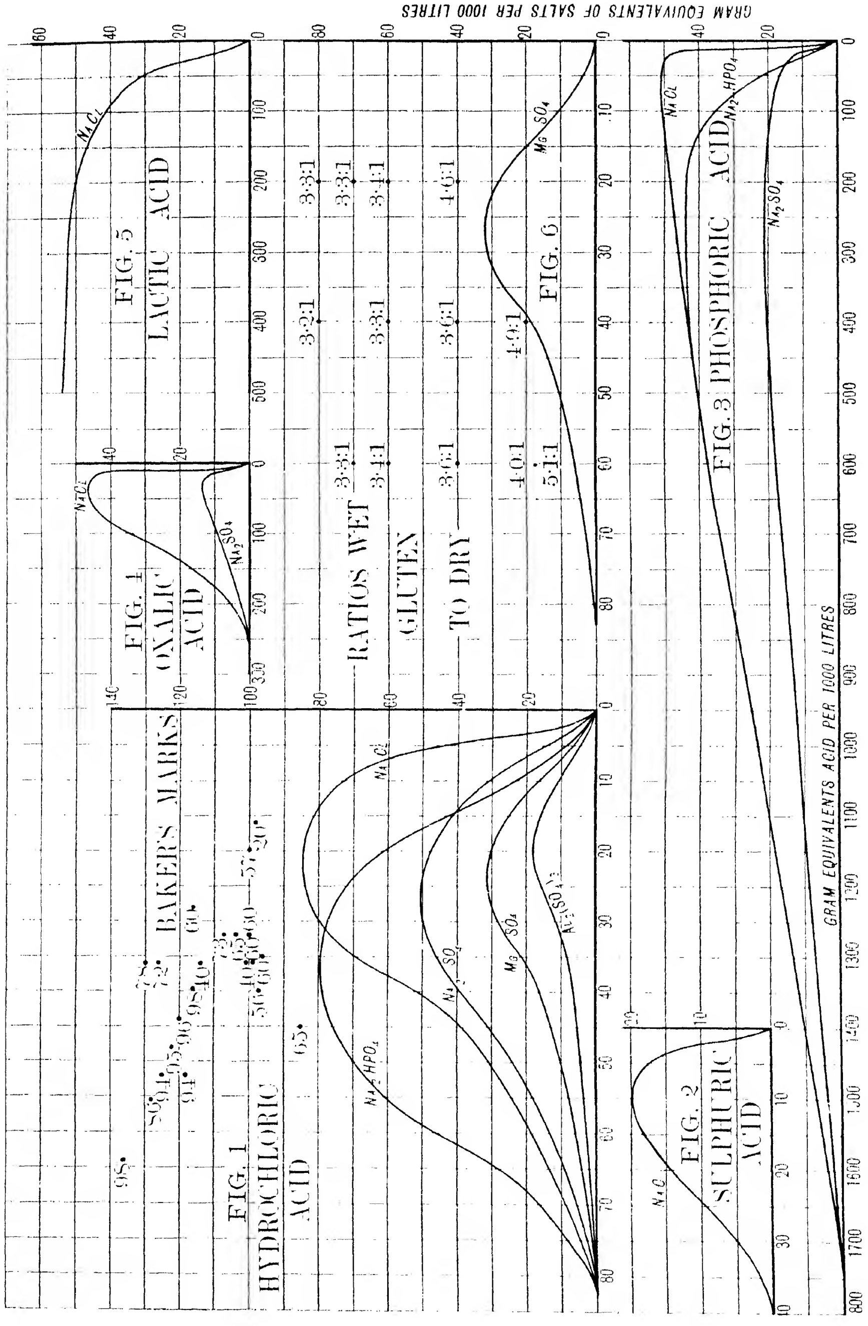
The experiments described above show quite clearly that the physical properties of gluten are entirely altered by changing its surroundings. Thus for concentrations of acid and salt which fall when plotted inside the curves, gluten is in a powdery or flocculent condition, quite devoid of coherence. For concentrations which fall on the curves gluten is just on the point of changing from the flocculent to the coherent condition. For concentrations outside the curves it is coherent.

Another point to notice is that the connexion between the properties of gluten and the acidity and salt content of its surroundings is not one which would become evident by comparing analytical figures with bakers' marks, for the same effect is produced by say, N/1000 HCl and N/100 NaCl, by N/50 HCl and N/10 NaCl, and by N/20 HCl and N/20 NaCl. In other words the amount of soluble salt required to produce a certain degree of coherence at first increases with the acidity up to a maximum and then falls off again. This is at once evident from the shape of the curves in Pl. VI. Figs. 1, 2, 3 and 4, and it probably explains why no one has succeeded in connecting acidity and soluble salt content with baking properties.

It has already been stated that the curves represent the conditions of concentration of acid and salt at which the gluten is just beginning to be coherent. If a bit of gluten suspended from a glass rod in a solution whose concentration is represented by a point on one of the curves is removed from its solution after attaining equilibrium, its coherence is only just sufficient to enable it to bear its own weight. It is far inferior in coherence, toughness and elasticity to a sample of gluten freshly rubbed out from a sample of good flour. It therefore seemed necessary to investigate the change of properties of gluten in contact with solutions whose concentrations are represented by points at varying distances outside the curves. This was done by immersing bits of gluten in such solutions by the method already described, allowing them 48 hours to attain equilibrium, removing them from their solution, leaving them to drain for half-an-hour, and drying them to constant weight in the steam-oven. Proceeding in this way one can readily determine the ratio of the weight of gluten when wet to the weight when dry. For points immediately outside the curves this ratio is found to be approximately 5 : 1. As the concentration of the salt increases, and the point moves away from the curve, the gluten gets continuously drier, and the ratio more nearly approaches that of freshly washed-out gluten. Figures for gluten in contact with hydrochloric acid and magnesium sulphate solution are given in Pl. VI. Fig. 6. They seem to offer an explanation for the well-known difference in water absorbing capacity found in certain flours, and since the toughness of the gluten increases as the water content falls, to connect both water absorption and toughness of gluten with acidity and content of soluble salt.

The experiments above described suggest that the variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of the glutens themselves. These properties must undoubtedly have a direct bearing on the power which some flours possess of making shapely loaves. I suggest therefore that the factor of strength on which the shape of the loaf depends is the relation between the concentrations of acid and soluble salts in the flour.

The correctness of these ideas should be capable of being tested in two ways. The most direct way would be to determine the acidity and soluble salt content of a flour, and to alter one or both by the


GRAM EQUIVALENTS OF SALTS PER 1000 LITRES
GRAM EQUIVALENTS ACID PER 1000 LITRES
FIG. 1 HYDROCHLORIC ACID
FIG. 2 SULPHURIC ACID
FIG. 3 PHOSPHORIC ACID
FIG. 4 OXALIC ACID
FIG. 5 LACTIC ACID
FIG. 6
BAKER'S MARKS
RATIOS WET GLUTEN TO DRY

addition of acid, alkali, or salt. Treated and untreated portions of the flour could then be submitted to baking tests in order to discover if the treatment had altered the water absorbing capacity or the shape of the loaf. A little consideration will however show that no positive result is likely to be obtained in this way. It has already been stated that a small bit of gluten takes 48 hours to attain equilibrium with any solution in which it may be immersed. Bread is always in the oven in a much shorter time than this after the flour is first moistened. In order therefore to give any added acid, alkali, or salt a chance to modify the gluten, it would have to be added some 40 hours before the doughing was begun, and since it could not be expected to act until moistened, the treated flour would have to be kept moist for that length of time before the yeast was added. Such a proceeding would of course be impracticable, both for mechanical reasons, and because bacterial fermentation would intervene.. That portion of the flour which is used for the sponge might possibly be treated with more hope of success, since it is longer in contact with the water.

Another but more indirect way of testing the idea is to determine the acidity and soluble salt content of a number of flours of known baking properties, to calculate from these analytical figures the concentrations of acid and salts as they exist in contact with the gluten in its natural state in each flour, to plot these concentrations side by side with the curves already given, and to see if the baking properties of the flours agree with the position of the points with respect to the curves. Flours of good baking properties should give fairly dry tough elastic gluten, and the points representing the acidity and salt content of such flours should be situated a considerable distance outside the curves, say in Pl. VI. Fig. 1. Weak flours on the other hand should give sloppy gluten with very little toughness and elasticity, and the points representing the acidity and salt content of such flours should fall only just outside the curves.

The experimental work necessary to make a number of such comparisons looks at first sight quite simple, and will I have no doubt ultimately turn out to be so, consisting simply of making a water extract of the flour under standard conditions, titrating a measured volume of this extract with a dilute standard alkali and phenolphthalein as indicator, and evaporating and igniting a second measured volume. But before the figures so obtained can be used to fix a position on the diagram which will indicate the kind of gluten the flour possesses, several important points must be settled.

The suggestion above made is, that the properties of gluten depend on the concentration of acid and salts, and on the kind of acid and salts, in the solution with which the gluten is in contact. The points which have to be settled therefore are: firstly, the nature of the acid and of the soluble salts contained in flour, and, secondly, the factor to be used to calculate, from the observed acidity and soluble salt content of the flour, the concentration of the solution upon which the properties of the gluten depend.

I am not yet in a position to make definite statements on either of these points. I have not up to the present attempted to investigate the nature of the acid. As regards the salts, I find that good flours of the Fife class contain in their soluble salts a preponderance of phosphate, whilst flours like Rivett contain little phosphate and much chloride. This point will be further investigated as soon as flours of the 1907 crop come to hand, and there should be no difficulty, other than experimental trouble, in settling it.

In the second point the difficulty is rather theoretical than experimental. It is quite simple to determine the percentages in the flour, but it is at present by no means clear how to convert these figures into the required concentrations. Before this can be done it must be decided at what stage the acid and salts influence the gluten so as to impress upon it the physical properties which decide the character of the flour. I take it that this must occur when the endosperm is being formed, at which time the grain contains much more water than when it is ready to grind. What is really required therefore is the concentration of the cell-sap of the grain during the process of endosperm formation. I hope to be able to determine this later, but for the present I have adopted the method of multiplying the percentages in the flour by the arbitrary factor 2, which corresponds to a water content in the endosperm of about 40 per cent. That this is not far from the truth is indicated by the fact that points calculated on this assumption are invariably situated on the diagram (Pl. VI. Fig. 1) in a region where the ratio of wet to dry gluten, which is predicted by the position of the point, agrees approximately with that found by experiment.

Working in this way a number of flours were examined, and the points corresponding to the acidity and salt concentration of the cell-sap of their endosperm are plotted on Pl. VI. Fig. 1. Each point is marked by a dot, to the left of which are printed the bakers' marks of the flour. It will be seen at once that the points corresponding to the

stronger flours fall much further outside the curves than the points corresponding to the weaker flours. Closer agreement than that shown cannot be expected until the debateable points already discussed have been worked out, and a method has been devised for measuring the shape of the loaf as distinguished from strength. The curves in the figure are those for hydrochloric acid and various simple salts. It is obvious that a much better comparison would be possible with curves for mixtures of salts made to imitate the soluble salts of various flours. In considering these figures it must be remembered that the bakers' marks express something like the mean of size and shape of loaf, while the position of a point on the diagram indicates the physical state of the gluten which can only influence one of these factors, the shape of the loaf.

I fully realise that my results are at present only in what may be called a suggestive state. My excuse for publishing them at once is that flours of the 1906 crop are no longer in a fit state to work upon, and several months must elapse before those of the 1907 crop are ready. In the meantime the results at least suggest a new method of investigating many practical problems in which the physical properties of proteid substances form an essential point.

Such a problem presents itself in the manipulation of the curd in cheese making. I suggested to my pupil Mr W. J. Richardson, B.A., that he should carry out some preliminary experiments on this subject. He has found the following method of working most satisfactory, and has obtained some interesting results which are given below.

To 200 c.c. of fresh separated milk contained in a flat dish about 1 inch deep, 10 c.c. N/10 lactic acid and 2 c.c. commercial rennet are added, the dish being kept for one hour in a water bath at 45° C. The block of curd so obtained is turned out, and cut into 3/4 inch cubes with a sharp knife. These cubes of curd are then immersed in small beakers containing acid and salt solutions of varying concentration as already explained in the case of gluten. Experimenting in this way, it is found that the curd remains coherent in water, and in very dilute acids, but begins to disintegrate in hydrochloric acid at N/300, and in lactic acid at about N/50. In the case of hydrochloric acid the rate of disintegration increases with the concentration up to about N/55, when it attains a maximum. Further increase in the concentration slows the rate of disintegration, until the curd remains coherent again at a concentration of about N/10. In the case of lactic acid, the rate of disintegration increases continuously with the concentration,

and no practicable concentration could be found which would again produce coherence.

This behaviour of hydrochloric and lactic acids on casein is exactly similar to their behaviour with gluten, and this similarity is made all the more striking when the action of salts in conjunction with these acids is investigated. Mr Richardson has roughly measured the concentration of salt required to produce coherence with varying concentrations of hydrochloric and lactic acids, his critical point being that concentration which made the curd cohere just enough to enable it to be picked out of the beaker with a glass rod.

He finds with hydrochloric acid that as the concentration is raised from N/300 to N/50, the concentration of sodium chloride required to preserve the coherence of the curd increases from the merest trace to N/10. For concentrations of HCl between N/50 and N/30, the concentration of NaCl remains constant at about N/10. As the concentration of HCl rises from N/30 to N/10, the concentration of NaCl falls from N/10 to zero. These results when plotted make a curve almost exactly like that for gluten in NaCl and HCl in Pl. VI. Fig. 1. For lactic acid, increasing concentrations of acid require increasing additions of salt to preserve coherence, and the curve obtained by plotting the results is of exactly the same kind as that for gluten in lactic acid and NaCl in Pl. VI. Fig. 5. Furthermore, the water content of the curd, as determined by drying to constant weight blocks drained for half-an-hour on tightly stretched muslin, varies in much the same way as the water content of glutens. Thus in one set of experiments, curd in equilibrium with a solution whose concentration of acid and salt came just inside the curve contained 93 per cent. of water. By increasing the salt until the point came just outside the curve, the water content fell to 91 per cent.

In another experiment, using hydrochloric acid and no salt, curd in equilibrium with N/1000 HCl contained 81 per cent. of water, as compared with 91 per cent. when in equilibrium with N/10 HCl, and 78 per cent. in N/2 HCl.

These experiments show that the consistency and water content of curd depends on the concentration of the acid and salt solution with which it is in contact.

Before concluding, I take this opportunity of once more expressing my thanks to Mr A. E. Humphries for supplying me with numberless samples of flour of known baking properties, to Mr G. G. Chapman,

B.A., of Peterhouse, and to Mr A. G. Simpson, B.A., of Trinity College, who have made many analyses and gluten tests for me, and to Mr W. B. Hardy, M.A., F.R.S., who has helped me all through the work with advice and suggestions.

Summary.

It is shown that the properties of gluten depend on the nature and concentration of acid and salts in the solution with which it is in contact, and that the connexion between the properties of gluten and the concentration of acid and salts is a peculiar one which would not be made evident by comparison of analytical figures with bakers' marks. This connexion is shown by curves in Pl. VI. Figs. 1—5.

The properties of gluten which vary with concentration of acid and salt are coherence, elasticity, and water content, and it is suggested that these properties have an important bearing on the shape of the loaf, and that a knowledge of the acidity and soluble salt content of a flour gives a clue to the factor of strength which decides whether the flour will make a good shaped loaf.

Finally it is suggested that the method of investigation adopted may be expected to throw light on all problems depending on the manipulation of proteids, cheese making being especially mentioned.

NOTE ON IMMUNE WHEATS.

By ALBERT HOWARD, M.A., F.L.S., *Imperial Economic Botanist to the Government of India*, AND GABRIELLE L. C. HOWARD, M.A., *formerly Fellow of Newnham College, Cambridge.*

WHILE reading the two interesting papers[1] on immune wheats in the last number of this *Journal* it occurred to us that our observations on rust resistant wheats in India might be of some interest. During the past two years a large number of varieties of Indian and European wheats have been grown by us at Pusa in Behar and at Lyallpur in the Punjab, one of the objects being to obtain wheats immune or at least resistant to rusts which would serve as parents in raising rust resistant hybrids. Biffen last year suggested that a trial should be made with Einkorn (*Triticum monococcum vulgare,* Kcke.), and kindly supplied us with a sample of the seed. Another sample was obtained from Messrs Vilmorin, and the sowings were made at the ordinary time, in October 1906 at Pusa and in November of the same year at Lyallpur. In all cases the grains germinated and produced the characteristic thick grass-like tufts of foliage but in no instance were ears formed, the plants remaining in the vegetative condition up to harvest time (March 1907 at Pusa and May 1907 at Lyallpur). Consequently no use could be made of Einkorn as a parent.

The behaviour of this variety towards rust proved however to be of interest. At Lyallpur, the leaves remained uniformly green till the hot weather set in towards the end of April, when it was found that numerous light-green translucent spots were being formed in the leaves. These were no doubt produced by the entry of infecting tubes of rust spores through the stomata into the intercellular spaces of the leaves resulting in the breaking down of the host cells in the manner described by Miss Marryat in the paper referred to above. Only in one case was a pustule observed and the development of this was extremely feeble.

[1] Biffen, *Journal of Agricultural Science*, Vol. II. p. 109; Marryat, *ibid.* Vol. II. p. 129.

Nothing further was noted up to the first week in May when the hot winds began to wither up the plants. As regards immunity to rust the behaviour of Einkorn in the Punjab closely corresponds with that observed at Cambridge and elsewhere. There was no lack of infecting material as the wheats in the surrounding plots and also in the vast stretches of country for miles round in the Chenab Colony were affected by both *Puccinia glumarum*, Eriks. and Henn., and to a less extent by *Puccinia graminis*, Pers. The weather towards the end of the wheat-growing season was very wet and cloudy and distinctly favourable to rust attack.

At Pusa the results were quite different. Up to the harvest time no pustules were formed on the leaves and the plot was immune to all three rusts *Puccinia triticina*, *P. glumarum*, and *P. graminis*, which were abundant on some or other of the numerous varieties grown close by. The light-green translucent spots on the leaves mentioned above were however produced, and it was decided to allow the plot to remain after harvest time to see if any ears would be formed. Early in May it was found that vigorous uredospore pustules were produced in large numbers on the leaves and this continued through the month and into June, when the pustules began to darken through the copious formation of teleutospores. Examination of the pustules and spores showed that they belonged to the black rust of wheat (*Puccinia graminis*). Uredospores were noticed up to June 15th when the plot had to be transplanted. April and May are the hottest months of the year in the Indo-Gangetic plain, and at Pusa this year the maximum shade temperature in these months varied from 84·2° F. to 105° F. We have here an interesting result of the struggle between the host and the parasite in the case of a plant ordinarily immune to a fungus. Perfect immunity was enjoyed by the host till the hot weather of April and May lowered its vitality to such an extent that pustules were formed in large numbers just as in the case of a wheat susceptible to rust attack. The formation of uredospores during the hot season is also of interest in connexion with the way in which wheat rusts pass over from one wheat crop to another in India. At present we are quite in the dark as to the way in which the wheat rusts survive the hot weather and monsoon in India and infect the crop in the following cold season. The behaviour of Einkorn towards *Puccinia graminis* at Pusa during the present hot weather indicates the possibility that if a suitable host plant were available this fungus might pass from one wheat crop to another in the uredo stage.

Although Einkorn did not prove of service as a rust resistant parent we were more fortunate with several varieties of Emmer[1] (*Triticum dicoccum*, Schrk.) which proved to be immune to the rusts met with at Lyallpur. These varieties flowered at the same time as the majority of the Indian wheats and reciprocal crosses were successfully made with several local wheats valuable in most respects but susceptible to rust attacks. The behaviour of Emmer towards rusts at Lyallpur closely followed that described by Miss Marryat in the case of Einkorn at Cambridge. Small circular sharply defined translucent spots and brownish-red dead areas surrounded by healthy green tissue were abundant on the leaves, but pustules were only very rarely produced and when found were very feebly developed. Side by side the wheats of the country were suffering from an epidemic of yellow rust (*Puccinia glumarum*). We hope that Emmer may be almost as useful in wheat breeding in certain parts of India as Einkorn has proved itself to be in Biffen's hands at Cambridge.

[1] The Indian varieties of Emmer are often referred to as Spelt wheats. We have however not yet discovered any Spelt wheats in India. As Spelts are mostly winter wheats it is hardly likely that they occur in India where the growing period of the crop is so short.

MENDELIAN HEREDITY IN COTTON.

By F. FLETCHER, B.A.

Indian Department of Agriculture.

In Volume II. Part 2 of this *Journal* there appeared a note by W. L. Balls on the above subject, in which he states that "long lint is completely dominant over short." This dominance, the present writer in India has found to occur in the case of crosses between many very different varieties. The question is not however so simple as it would appear to be from Mr Ball's statement "that the breeding of pure types suitable to the needs of the manufacturer and the cultivator will possibly prove a little difficult"; for though length of staple is on the whole dominant we often get seeds that carry both long and short cotton. This is the case in some of the long-established American varieties, for instance "Bragg Long Staple" produces cotton of which almost the whole has a length of about $1\frac{3}{8}$ inches, but some of the seeds carry cotton both of this length and also of over twice the length, the latter inherited from its Sea-Island parent.

"Mixed staple" of this character I have repeatedly met with in breeding experiments, and its occurrence has terminated the career of more than one otherwise very promising hybrid.

With regard to the inheritance of the colour of the flower, this is in certain cases complex as Mr Balls states. Thus if a red-flowered variety be crossed with a yellow-flowered one, red- and yellow-flowered plants appear in about equal numbers in the F_1 generation. In the F_2 generation, I got of the red-flowered hybrid 428 giving rise to red-flowered plants and 114 to yellow, while of the yellow F_1 generation 12 only gave red to 474 yellow.

The above statement applies also to cases in which the red (or purple) colour is not diffused over the whole of the petal, but is confined to its base. Thus American (Upland) crossed with Sea-Island gave in the F_1 generation 11 plants without a purple "eye,"

16 with a faint one, and 8 with one as deep as the present Sea-Island.

In crossing yellow- and white-flowered varieties, however, we get complete dominance of the yellow.

Again some varieties have a short "fuzz" which completely covers the seeds after the removal of the cotton, while in others the seed is naked. The presence of fuzz appears to be completely dominant.

Some varieties differ from others only in that the cotton comes away from the seed much more easily. This looseness of the cotton on the seed also appears to be dominant.

The pairs of characters so far investigated are:

	Dominant	Recessive
Cotton	Fineness	Coarseness
	Length	Shortness
	Colour	Whiteness
Petal	Yellow colour	White
Seed	Fuzziness	Nakedness
	Loose cotton	Adhering cotton
Plant	Lateness	Earliness

Unfortunately earliness appears to be coupled[1] with shortness of fibre, and it is doubtful how far earliness can be secured except at the expense of quality.

In the character of the leaves, shape of the bolls and habit of the plant, all shades intermediate between the parents are found in the F_1 generation.

I agree with Mr Balls in his opinion as to the evils resulting from the presence of "weeds" in the Egyptian cotton fields—one of which is the *G. Figarei* of Todaro, once grown as a field crop in Egypt under the name "Hamuli." In attempts to improve Egyptian cotton, it is necessary first to exclude the influence of this variety.

[1] Another case of coupling is that between the colour of the cotton and its weakness.

THE BOTANICAL AND CHEMICAL COMPOSITION OF THE HERBAGE OF PASTURES AND MEADOWS.

By S. F. ARMSTRONG, Univ. Dipl. Agric.,
Cambridge University Department of Agriculture.

INTRODUCTORY NOTE[1].

In view of the great importance of grazing in Great Britain, and especially in view of the increasing areas which have been laid down to grass during the last thirty years, it is remarkable that so little attention has been given to the composition of the herbage of pastures. Analyses of the mixed herbage produced in hay fields have frequently been made, but these give little information on the point at issue, for it is obvious that under the very different conditions existing in pasture and meadow, the balance of advantage in the struggle for sunlight and soil will probably rest with entirely different species in the two cases.

The most valuable information on the subject is that given by the work of the late Dr W. Fream[2], whose method of investigation was as follows: turfs two feet long, one foot wide, and nine inches deep were collected from 80 localities in the British Isles, and planted in the botanic garden of the College of Agriculture, Downton.

The herbage of these turfs was allowed to grow until ready to harvest, when it was clipped off with shears, and its composition ascertained by botanical separation. His figures thus show the composition of hay made from pastures and harvested in July, but they cannot be relied on to give anything like an accurate indication of the actual composition of the herbage available for stock grazing.

In order accurately to ascertain the composition of the herbage of a pasture at any particular season, it would be necessary to remove all edible herbage, and to separate and weigh the different species. This method however is so tedious as to be impracticable for a general survey, and it occurred to me that by measuring the relative proportions of ground surface occupied by the different species on a number of

[1] By T. H. Middleton, M.A.

[2] *J. R. A. S. E.* 2nd Series, xxiv. 415; 3rd Series, i. 359.

small representative areas, a good approximation to the true composition of the herbage might be obtained.

Mr Armstrong has applied this process to a number of examples of several types of pasture and meadow land, notably to some of the excellent old pastures in the Market Harborough district of Leicestershire and Northamptonshire. The results of his investigation are set forth in the following pages, together with chemical analyses of the soils and of the herbage, made in the University Agricultural Laboratory by Messrs F. W. Foreman, A.I.C., assisted by G. G. Chapman, B.A.

T. H. M.

Objects of the Investigation.

The principal objects in view were:

1. To determine the botanical composition of the herbage of some of the choicest grazing land in the district, and to see in what respects its herbage differed from that of second-class and inferior types of pasture in the same neighbourhood.

2. To find out how these same types of grass land differed in regard to the chemical composition of their soils and herbage.

3. To see what changes occurred in the botanical and chemical composition of the herbage during a growing season.

Methods employed.

Selection of Fields.

In selecting fields use was always made of the occupier's knowledge and experience. Thus some fields were chosen because for many years they had been noted for the number of bullocks which they could fatten off in an average season with but little artificial assistance. Other fields were examined because it was known that they were suitable only for grazing by store cattle or sheep. As we wished to compare different types of grazing land rather than individual fields, a portion only of each enclosure, some two or three acres in extent, and representing the type of pasture, was selected for examination. When two distinct types of herbage were found in the same field, each was examined separately. A list of the fields examined will be found in the annexed table—Table I—together with a very brief description of the soil, locality, and situation of each.

TABLE I. *The Herbage of Old Pastures. Names and description of Fields examined, arranged as far as possible according to quality and feeding value of their herbage.*

Ref. No.	Name of Field	Locality	County	Geological Formation	Soil	Situation and Aspect	Remarks
1	"The Seeds"	Slawston	Leicester	Upper Lias & Marlstone	Loam	High-lying and on S.W. slope exposed	Close to village, and has been well treated
2	Spence's Meadow	Do.	Do.	Marlstone	Clayey loam, deep, moist	Sheltered and level	Do., grazed and mown alternately
3	"Cow Close"	Do.	Do.	Lias	Moist, clayey loam	Do.	Very little cake fed on it
4	"Hall Close"	Welham	Do.	Do. and Alluvium	Deep and moist loam	Low-lying, flat	Lies against the river Welland
5	"The Old Churchyard"	Medbourne	Do.	Lias	Loam	High-lying on exposed southern slope	
6	"Round Pond" Field	Oxendon	Northants	Do.	Do.	Level	Have been laid down to grass about 30 years
7	Watson's "Seeds"	Little Bowden	Do.	Boulder clay	Clay	Situated on top of a hill	
8	"Church Field"	Oxendon	Do.	Do.	Heavy clay	North-east slope	No seeds mixture sown
9	"High Field"	Market Harboro'	Leicester	Lias	Loam	Gentle southern slope	
10	"The Grove"	Impington	Cambs.	Gault	Do.	Level, sheltered	
11	"Top Meadow"	Slawston	Leicester	Lias	Do.	Level	Grazed and mown alternately
12	"The Big Field"	Oxendon	Northants	Do.	Do.	High-lying, exposed southern slope	Old pasture with very close turf
13	"Washpit"	Ashley	Do.	Do.	Do.	Mostly low-lying	Two different types of soil and herbage
14	Unwin's "Old Pasture"	Impington	Cambs.	Gault	Dark loam	Level, sheltered	Very old pasture
15	"Solstaceway"	Slawston	Leicester	Lias	Loam	Level, open	Will not fatten bullocks
16	"Lane's End"	Thorpe Langton	Do.	Do.	Do.	Do.	Do.
17	"Middle Welham"	Welham	Do.	Do.	Do.	Level, sheltered	Grazed and mown alternately
18	"Clock Close"	Slawston	Do.	Do.	Do.	Low-lying and level	Soil suffers through excess of wetness; also badly treated
19	"Road Part"	Do.	Do.	Do.	Do.	Level	Soil thin, dry, and impoverished; grazed by sheep
20	Experimental Field, Plot 6	Cransley	Northants	Boulder clay	Clay	High-lying, but sheltered	A wretched pasture

Sampling of Soil and Herbage.

The soils were sampled by taking cores with a sampling tool from different places over a representative area. The analyses were made in duplicate. Herbage for chemical analysis was obtained by going over the same area and plucking off the mixed pasture plants with the thumb and forefinger. In this way about the same proportion of each plant was obtained as would be taken off by an ox in grazing. Duplicate samples were taken, wrapped in water-proof paper, labelled, and at once sent to the laboratory. The dry matter, nitrogen, and phosphate were determined in each sample, duplicate analyses being made. The duplicate samples taken as described were found to agree very satisfactorily in chemical composition.

Botanical Analysis of the Herbage.

As already stated in the introduction the botanical analyses of the herbage were made by estimating the proportion of ground surface occupied by each species. For this purpose a square frame was used which enclosed an area of one square foot. This space was further divided into square inches by means of cord tightly stretched across. After fastening the frame to the ground the number of square inches of bare surface, if any, were first counted. Then the larger and more conspicuous plants such as ryegrass, white clover, cocksfoot or weeds were similarly dealt with, and lastly the less abundant species. With constant practice and care the "total area" thus obtained did not differ from the actual area, 144 square inches, by more than three or four square inches which were added or subtracted proportionally. Frequently more than 80 per cent. of the surface was occupied by three or four species only.

A general idea of the herbage was first obtained and then a number of representative portions of the turf, usually from six to ten, according to the character of the herbage, were examined in this way. The average of these was taken to represent the approximate composition of the herbage at the time.

Results obtained in this way were compared with the percentages found by separating and weighing the approximate constituents of the herbage of several fields. The figures (Table III) indicate a close agreement between the results obtained by either method. That the method employed gives the composition of the pasturage far more accurately than that of finding the composition by weight of a hay crop is clearly shown by a comparison of the results given in Tables II and III.

TABLE II. *Showing, for example, in the case of three common pasture plants, how very different the percentages of each in the pasture, and in the hay crop may be.*

Place and Description	Abbotsley, 1905, New Pasture. Av. of eleven plots. Percentage in Pasture	Hay crop	Cransley, 1906, Old Pasture. Av. of five plots. Percentage in Pasture	Hay crop	Top Meadow, 1906, Very old grass land. Percentage in Pasture	Hay crop
Date	July	July	June	July	May	August
Trifolium repens	8·6	3·2	20·7	5·6	19·3	7·3
Lolium perenne	27·8 *	33·5 *	7·9	11·2	25·7	43·1
Cynosurus cristatus...	10·1 †	8·9 †	20·4	14·2	10·0	13·0

* Average of eight plots. These species were not sown on all the plots.
† ,, four plots. ,, ,, ,, ,,

TABLE III. *Comparison of the results of estimating the composition of herbage, (1) by weight, and (2) by the area occupied by each species.*

Figures give percentages.

Approximate constituents of herbage	* "The Grove" Area	Wt.	† "The Seeds" Area	Wt.	"Hall Close" Area	Wt.	"Old Churchyard" Area	Wt.	"Round Pond Field" Area	Wt.	‡ New Pasture, No. 19, Univ. Farm Area	Wt.
Total Grasses	56·6	59·8	56·3	56·3	61·4	61·3	55·1	57·7	50	49·5	46·9	42·8
White Clover.	32·0	30·0	42·1	42·5	36·3	36·0	43·9	42·3	50	50·5	46·3	56·3§
Yarrow.........	6·0	5·7	—	—	2·0	2·6	—	—	—	—	—	—
Miscellaneous	5·4	4·5	1·3	1·2	Tr	—	—	—	—	—	1·3	·9
Bare surface	—	—	·3	—	—	—	1·0	—	—	—	5·5	—
	100	100	100	100	99·7	99·9	100	100	100	100	100	100

* Average of four places. † Average of two places.
‡ Average of three places. The other fields one place each.

§ The rather large difference here is due to the removal of portions of clover runners which were thus included in the weight. On this type of pasture the proportion of creeping stems to foliage in the case of white clover was greater than on older turfs. They cannot be strictly regarded as edible herbage.

TABLE IV. *Rainfall recorded in the Market Harborough district.*

Monthly Rainfall.

October, 1905—September, 1906.

	Inches		Inches
1905		*1906*	
October	1·19	April	0·78
November ...	2·48	May	1·81
December ...	0·94	June	2·62
1906			
January......	3·66	July	1·69
February	2·22	August	1·26
March	1·46	September...	0·96
Winter rainfall	*11·95*	*Summer rainfall*	*9·12*
Average ,,	12·35	Average ,,	13·37
Diff. from Av.	– 0·40	Diff. from Av.	– 4·25

Weekly Rainfall.

Rainfall for each week from January 1st to July 28th, 1906. Also greatest rainfall recorded on any one day during the weekly period.

Successive weeks from January 1st	1906 Fall, Inches	1906 Max.	1906 Date	Successive weeks from April 16th	1906 Fall, Inches	1906 Max.	1906 Date
1	1·19	·62	January 6	16	0·18	·13	April 22
2	1·12	·48	,, 8	17	0·45	·25	,, 23
3	1·12	·38	,, 16	18	0·74	·22	May 2 and 3
4	0·23	·23	,, 24	19	0·22	·19	,, 7
5	0·27	·27	February 2	20	0·36	·18	,, 16 ,, 20
6	0·66	·66	,, 10	21	0·49	·29	,, 26
7	0·63	·24	,, 16	22	0·42	·30	June 1
8	0·17	·13	,, 19	23	0·00	—	—
9	0·71	·33	,, 27	24	0·56	·34	,, 15
10	0·48	·24	March 10	25	0·00	—	—
11	0·32	·28	,, 14	26	1·64 *	·82	,, 28 ,, 29
12	0·29	·19	,, 24	27	0·00	—	—
13	0·15	·15	,, 26	28	0·25	·10	July 10
14	0·15	·15	April 2	29	0·00	—	—
15	0·00	—	—	30	1·39	1·16	,, 27

* Measured in two successive days.

DESCRIPTION OF SEASON.

Table IV gives the rainfall recorded in the neighbourhood of Market Harborough during the time this work was in progress. The autumn of 1905 was cold with less than the average fall of rain. The season of 1906 was remarkable for the amount of bright sunshine recorded, but the mean temperature was not above the average. The total fall of rain for the twelve months—October 1905 to September 1906—was much below the average, and the deficiency occurred chiefly during the summer. It should be noticed that although 4·26 inches of rainfall was measured in June and July, of this amount 2·80 inches fell during three days. The dry weather during the latter part of June and throughout July was especially trying for the pastures.

DISCUSSION OF RESULTS.

BOTANICAL COMPOSITION OF HERBAGE[1].

a. First-rate Old Pastures.

The first three fields in Table V represent the richest type of old grazing land found in the Market Harborough district. In No. 1 annual meadow grass (*Poa annua*) was present in quite an unusual quantity, but otherwise the composition of the herbage on the three fields was very similar.

An average of these fields gives the following order of relative abundance of the more common species:

1. White Clover (*Trifolium repens*).
2. Ryegrass (*Lolium perenne*).
3. Crested Dogstail (*Cynosurus cristatus*).
4. Fiorin (*Agrostis alba* var. *stolonifera*).
5. Rough-stalked Meadow Grass (*Poa trivialis*).
6. Yorkshire Fog (*Holcus lanatus*).

The most striking figures in this table are the very high percentages of white clover and ryegrass which together formed more than two-thirds of the entire herbage. Fiorin was rather abundant, and also Yorkshire fog on No. 4. The low percentage of weeds consisted chiefly of the bulbous buttercup (*Ranunculus bulbosus*).

[1] N.B. The analyses quoted in comparing each type of herbage are always the average of the two examinations made during the summer of 1906 which are conveniently grouped in Table X. In Tables V—XI the figures give the percentage of the surface occupied by each species.

TABLE V. *Botanical Composition of the Herbage of four first-rate Old Pastures.*

Reference No.	1				3				4				14				
Name	"The Seeds"				"Cow Close," Section A.				"Hall Close," Section A.				"Unwin's Pasture"				
Date of Examination	Oct. 1905	May 24th, 1906	July 20th, 1906	*Average*	Oct. 1905	May 24th, 1906	Aug. 4th, 1906	*Average*	Oct. 1905	May 12th, 1906	Aug. 4th, 1906	*Average*	Sept. 1905	March 7th, 1906	April 27th, 1906	June 18th, 1906	*Average, excluding March 7th*
No. of places examined	4	8	10	*7*	6	8	10	*8*	8	6	8	*7*	4	4	5	4	*4*
Lolium perenne	36·4	35·0	37·5	*36·3*	43·8	28·1	33·4	*35·1*	38·9	24·2	37·0	*33·4*	47·5	52·0	35·4	35·0	*39·3*
Dactylis glomerata	·7	—	—	*·2*	·3	·8	—	*·4*	—	1·7	1·0	*·9*	3·4	4·1	3·6	6·4	*4·5*
Cynosurus cristatus	7·4	4·3	2·7	*4·8*	12·1	13·3	8·5	*11·3*	6·6	7·1	1·7	*5·1*	trace	·6	5·3	8·0	*4·4*
Alopecurus pratensis	·9	1·1	—	*·7*	·6	1·0	—	*·5*	—	—	—	—	—	—	1·1	—	*·4*
Phleum pratense	4·0	1·7	—	*1·9*	·7	1·5	trace	*·7*	1·9	3·4	2·0	*2·4*	trace	—	—	—	*trace*
Avena flavescens	—	—	—	—	—	—	—	—	·6	—	—	*·2*	—	·6	2·2	2·1	*1·4*
Poa trivialis	1·4	4·0	1·8	*2·4*	·8	4·4	1·5	*2·2*	5·5	4·9	1·3	*3·9*	4·9	3·4	2·6	2·3	*3·3*
Poa annua	8·2	11·8	12·8	*10·9*	—	—	trace	*trace*	2·1	1·8	trace	*1·3*	—	—	—	—	—
Festuca ovina et vars.	—	—	—	—	·5	trace	—	*·2*	·5	1·2	·7	*·8*	12·5	5·0	6·8	13·4	*10·9*
Agrostis alba, var. stolonifera	12·8	4·0	4·3	*7·0*	5·4	4·5	4·5	*4·8*	11·2	7·6	11·3	*10·0*	—	2·0	1·3	trace	*·4*
Holcus lanatus	1·0	1·2	1·2	*1·1*	2·3	2·4	2·0	*2·2*	17·2	5·0	6·5	*9·6*	—	—	—	—	—
Hordeum pratense	—	—	—	—	—	—	—	—	—	—	—	—	3·4	—	—	1·6	*1·7*
Aira cæspitosa	—	—	—	—	—	—	—	—	1·1	—	—	*·4*	—	—	—	—	—
Trifolium pratense	—	—	—	—	1·3	—	trace	*·4*	—	—	—	—	—	—	·1	—	*trace*
Trifolium repens	24·6	35·5	38·9	*33·0*	26·2	39·7	47·5	*37·8*	12·3	40·3	36·6	*29·7*	12·0	6·2	13·4	13·3	*12·9*
Ranunculus sps.	·9	1·4	—	*·8*	2·2	3·2	trace	*1·8*	1·2	2·5	1·1	*1·6*	4·1	8·2	8·7	2·9	*5·2*
Bellis perennis	—	—	—	—	—	—	—	—	·3	—	—	*·1*	10·4	10·5	13·5	8·1	*10·7*
Taraxacum officinale	·9	—	—	*·3*	—	—	—	—	·6	—	—	*·2*	1·3	2·0	3·6	—	*1·6*
Mosses	—	trace	—	*trace*	2·6	1·0	trace	*1·2*	—	—	—	—	trace	4·1	·5	1·2	*·6*
Various weeds	·7	trace	·4	*·4*	·4	trace	trace	*·2*	—	trace	trace	*trace*	—	—	·6	2·0	*·9*
Totals	99·9	100·0	99·6	*99·8*	99·2	99·9	97·4	*98·8*	100·0	99·7	99·2	*99·6*	99·5	98·7	98·7	96·3	*98·2*
Gramineae	72·8	63·1	60·3	*65·3*	66·5	56·0	49·9	*57·4*	85·6	56·9	61·5	*68·0*	71·7	67·7	58·3	68·8	*66·3*
Leguminosae	24·6	35·5	38·9	*33·1*	27·5	39·7	47·5	*38·2*	12·3	40·3	36·6	*29·7*	12·0	6·2	13·5	13·3	*12·9*
Weeds	2·5	1·4	·4	*1·4*	5·2	4·2	trace	*3·2*	2·1	2·5	1·1	*1·9*	15·8	24·8	26·9	14·2	*19·0*

NOTE. "Unwin's Pasture"—a small enclosure near buildings—is placed here as representing pasture of good quality for the neighbourhood of Cambridge. It is, however, much inferior to fields Nos. 1, 3 and 4.

Table VI.

Botanical Composition of the Herbage of four Excellent Recent Pastures.

Reference No.	5		7		6		8	
Name	"The Old Churchyard"		"Watson's Seeds"		"Round Pond Field"		* "Church Field"	
Date of Examination, 1906	June 8th	July 31st	June 8th	July 24th	June 5th	July 26th	June 5th	July 26th
No. of places examined ...	8	10	8	10	10	10	8	10
Lolium perenne	31	32·5	28	47·2	32·1	35·7	30·7	41·3
Dactylis glomerata	4	4·8	12	7·0	2·3	1·5	1·1	1·0
Cynosurus cristatus	10	6·0	11	2·8	14·6	10·0	10·1	9·4
Alopecurus pratensis	—	—	trace	—	—	trace	trace	trace
Phleum pratense............	2	trace	trace	—	1·8	trace	3·7	trace
Avena flavescens............	trace	trace	1	·7	—	trace	·7	—
Poa trivialis..................	6	6·6	11	3·5	11·8	1·7	12·8	2·9
Poa pratensis	1	trace	trace	trace	—	—	·6	—
Poa annua	4	5·0	2	2·3	2·2	3·5	·7	1·6
Festuca pratensis	1	1·0	—	—	—	—	—	—
Festuca ovina	—	trace	1	·5	·4	trace	1·8	trace
Agrostis alba, var. stolonifera	trace	trace	trace	trace	2·5	5·2	1·8	4·5
Holcus lanatus	1	trace	5	1·2	2·8	2·0	6·6	2·2
Agropyrum repens	trace	—	—	trace	trace	—	trace	—
Trifolium repens............	37	40·3	21	27·8	29·5	38·6	26·5	35·1
Trifolium pratense.........	—	—	trace	trace	—	—	—	—
Ranunculus sps.............	—	—	†	†	trace	trace	·5	·5
Taraxacum officinale	—	—	†	†	—	—	—	—
Bellis perennis	—	—	†	†	trace	—	·7	·8
Plantago lanceolata	—	—	†	†	—	—	—	—
Carduus sps.	—	—	†	†	—	—	—	—
Mosses	—	—	†	†	trace	—	·7	—
Various weeds..............	—	—	—	—	trace	trace	·4	—
Bare surface..................	—	·5	—	·7	—	·6	—	—
Totals	98·0	96·7	99·5	98·4	100·0	98·8	99·4	99·3
Gramineae	60·0	55·9	71·0	65·2	70·5	59·6	70·6	62·9
Leguminosae	37·0	40·3	21·0	27·8	29·5	38·6	26·5	35·1
Weeds	1·0	trace	7·5	4·7	trace	trace	2·3	1·3

* "Church Field," Oxendon, was not sown down, but went to grass naturally.

† Denotes that the quantity of the species present was not ascertained separately, but is included in the totals at the base of the table.

"Unwin's Pasture" (No. 14), selected as being one of the best near Cambridge, is of quite a different type from the foregoing. It has an old and very thick turf growing on a dark loam. The figures show that weeds—especially daisies (*Bellis perennis*)—were abundant, and that sheep's fescue (*Festuca ovina*) was a conspicuous grass. Even here, however, ryegrass and white clover occupied about one-half of the surface.

b. *Excellent Recent Pastures.*

It was ascertained that some of the finest grazing land in the Market Harborough district had been formed about thirty years ago. Table VI gives the composition of the herbage of four of these recent pastures, which were considered to be equal to the best old grazing land. Taking an average of these fields, it is seen that ryegrass and white clover again formed about two-thirds of the herbage. Compared with the best old pasture fiorin was less common, while crested dogstail, cocksfoot (*Dactylis glomerata*) and rough-stalked meadow grass were rather more abundant. Nos. 5, 6 and 8 were remarkably free from weeds.

c. *Second-rate Old Pastures.*

Pasture of good feeding quality, but not considered first-class for the district, is represented by the fields in Table VII. Compared with the best old pastures (see Table X) the chief botanical difference in the herbage was the diminished quantity of white clover present. On the whole ryegrass, crested dogstail, and weeds were rather more abundant, but the slight falling off in quality appeared to be due rather to the condition of the soil than to the species composing the herbage.

d. *Inferior Old Pastures.*

Fields Nos. 15, 16 and 19 (Table VIII) represent the inferior grazing land of the neighbourhood. The herbage of these pastures presents a remarkable contrast to the fields in Tables V and VI. Looking at the averages of "Road Part" and "Solstaceway," it will be seen that in both cases fiorin (chiefly *Agrostis vulgaris*) and weeds occupied between 40 and 50 per cent. of the surface. Ryegrass and white clover took quite secondary places. The former occupied on the average only one-half and the latter only one-fifth of the proportion of surface which they filled in the best old pastures.

TABLE VII. *Botanical Composition of the Herbage of three Old Pastures*—which were considered to be of very good feeding quality, though not first-rate.

Reference No.	9			12			13		
Name	"Highfield"			"The Big Field"			"Washpit" (Section C)		
Date of Examination, 1906	May 31st	July 24th	*Average*	June 6th	July 30th	*Average*	June 7th	July 28th	*Average*
No. of places examined ...	8	10	*9*	6	10	*8*	6	6	*6*
Lolium perenne	38·8	53·4	*46·1*	30·2	36·0	*33·1*	19·3	36·2	*27·6*
Dactylis glomerata	2·3	1·7	*2·0*	—	—	—	3·2	1·6	*2·4*
Cynosurus cristatus	6·7	3·5	*5·1*	17·4	13·5	*15·5*	13·3	3·7	*8·5*
Alopecurus pratensis	—	trace	*trace*	—	trace	*trace*	—	trace	—
Phleum pratense............	4·2	1·0	*2·6*	2·0	·7	*1·3*	1·9	1·4	*1·6*
Avena flavescens............	2·7	trace	*1·4*	—	—	—	4·5	2·2	*3·3*
Poa trivialis..................	10·7	·9	*5·8*	5·1	·7	*2·9*	2·9	1·0	*1·9*
Poa annua....................	·7	—	*·3*	1·7	2·8	*2·2*	—	—	—
Anthoxanthum odoratum	—	—	—	3·4	2·5	*3·0*	—	—	—
Festuca ovina	·7	1·0	*·9*	trace	—	*trace*	7·8	7·2	*7·5*
Agrostis alba, var. stolonifera	7·3	5·7	*6·5*	4·0	5·3	*4·6*	1·7	6·6	*4·1*
Holcus lanatus	trace	1·4	*·7*	11·0	6·9	*9·0*	2·8	2·3	*2·6*
Trifolium repens............	20·4	27·5	*24·0*	21·4	30·9	*26·0*	18·9	28·8	*23·8*
Achillea Millefolium	—	trace	*trace*	—	—	—	1·9	trace	*·9*
Ranunculus sps.	4·3	2·0	*3·1*	2·0	trace	*1·2*	5·5	trace	*2·8*
Taraxacum officinale	—	—	—	—	—	—	*	*	*
Bellis perennis	—	—	—	—	—	—	9·5	1·1	*5·3*
Plantago lanceolata	—	—	—	—	—	—	*	*	—
Mosses	—	—	—	1·0	trace	*·6*	*	trace	*trace*
Various weeds	1·1	—	*·5*	·7	trace	*trace*	6·8	5·3	*6·0*
Bare surface..................	—	1·0	*·5*	—	trace	*trace*	—	1·7	*·8*
Totals	99·9	99·1	*99·5*	99·9	99·3	*99·4*	100·0	99·1	*99·1*
Gramineae	74·1	68·6	*71·4*	74·8	68·4	*71·6*	57·4	62·2	*59·5*
Leguminosae	20·4	27·5	*24·0*	21·4	30·9	*26·0*	18·9	28·8	*23·8*
Other useful Plants.........	—	trace	*trace*	—	—	—	1·9	—	*·9*
Weeds	5·4	2·0	*3·6*	3·7	trace	*1·8*	21·8	6·4	*14·1*

NOTES. No. 9 was the field examined by Lawes and Gilbert on several occasions between 1879 and 1888. Its quality has somewhat deteriorated in recent years.

An asterisk shows that the quantity of the species was not ascertained separately, but that it has been included in the totals at the foot of the table.

TABLE VIII. *Botanical Composition of the Herbage of four inferior Old Pastures.*

Reference No.	19			15				16	20
Name of Field	"Road Part"			"Solstaceway"				"Lane's End"	Plot 6, Cransley
Date of Examination, 1906	May 14th	Aug. 1st	*Average*	Oct. 1905	May 21st	Aug. 1st	*Average, May, Aug.*	Oct. 1905	June 25th, 1906
No. of places examined ...	6	8	*7*	6	4	4	*4*	6	6
Lolium perenne	12·4	15·7	*14·1*	39·4	18·0	18·0	*18·0*	44·6	1·0
Dactylis glomerata	9·9	9·1	*9·5*	5·9	1·6	trace	*·9*	3·0	3·4
Cynosurus cristatus	8·5	5·4	*7·0*	14·8	18·2	7·0	*12·6*	13·6	18·9
Alopecurus pratensis	—	—	—	·6	1·3	—	*·6*	—	—
Phleum pratense	—	—	—	1·0	1·3	—	*·6*	—	—
Avena flavescens	1·5	trace	*·8*	1·5	3·7	1·3	*2·5*	1·1	2·0
Poa trivialis	—	trace	*trace*	—	trace	—	*trace*	·7	—
Poa pratensis	—	—	—	—	—	—	—	—	trace
Anthoxanthum odoratum	·8	1·2	*1·0*	—	2·6	·8	*1·7*	—	—
Festuca ovina et vars. ...	3·2	3·7	*3·4*	1·3	2·0	2·7	*2·3*	1·4	5·8
Festuca pratensis	—	—	—	—	—	—	—	—	trace
Agrostis alba et vars.	17·4	19·5	*18·5*	14·8	22·7	51·8	*37·2*	13·5	trace
Holcus lanatus	1·0	1·2	*1·1*	6·0	1·3	1·4	*1·3*	·8	3·0
Agropyrum repens	1·3	—	*·6*	—	—	—	—	—	1·2
Trifolium minus	—	—	—	—	—	—	—	—	1·7
Trifolium repens	3·4	6·8	*5·1*	3·7	9·3	14·0	*11·6*	10·2	6·0
Trifolium pratense	—	1·7	*·8*	2·3	—	—	—	2·4	—
Lotus corniculatus	7·0	6·2	*6·6*	1·0	·7	trace	*·4*	—	—
Medicago lupulina	—	—	—	—	—	—	—	—	15·0
Achillea Millefolium	1·9	5·8	*3·8*	—	—	—	—	—	—
Ranunculus sps.	8·9	*	*	2·4	10·2	—	*5·1*	3·5	trace
Taraxacum officinale	·9	*	*	—	1·3	—	*·6*	1·1	—
Bellis perennis	3·6	*	*	·8	—	—	—	1·3	11·4
Plantago lanceolata	2·7	*	—	·4	—	1·0	*·5*	trace	6·2
Luzula campestris	2·5	trace	—	—	1·7	—	*·8*	—	·6
Leontodon sps., Hieracium sps., Hypochœris sps.	7·3	13·4	*10·3*	—	2·5	2·0	*2·2*	—	10·2
Mosses	1·4	1·4	*1·4*	—	—	—	—	1·7	2·2
Carex glauca	—	—	—	—	—	—	—	—	7·6
Ajuga reptans	—	—	—	—	—	—	—	—	1·3
Various weeds	1·0	*	*	1·2	—	—	—	1·0	trace
Bare surface	1·3	1·3	*1·3*	·6	—	—	—	—	—
Totals	97·9	96·6	*97·2*	97·7	98·4	100·0	*98·9*	99·9	97·5
Gramineae	56·0	55·8	*56·0*	85·3	72·7	83·0	*77·7*	78·7	35·3
Leguminosae	10·4	14·7	*12·5*	7·0	10·0	14·0	*12·0*	12·6	22·7
Other useful Plants	1·9	5·8	*3·8*	—	—	—	—	—	—
Weeds	28·3	19·0	*23·6*	4·8	15·7	3·0	*9·2*	8·6	39·5

* See note, Table X.

Crested dogstail was more abundant than on the better fields. Birdsfoot trefoil (*Lotus corniculatus*), a plant typical of impoverished soils, was plentiful on "Road Part."

The herbage of "Lane's End" was very similar to that of "Solstaceway," and was only examined once.

Plot 6, Cransley, near Kettering, represents for comparison an extreme type of poor pasture. The large quantities of weeds, crested dogstail and medick (*Medicago lupulina*), and the extremely low percentages of ryegrass and white clover should be noted.

e. *Meadows.*

With each of the four meadows in Table IX the usual practice was to graze and mow in alternate years.

No. 2 represents the choicest meadow land of the neighbourhood. It is grazed by dairy cows and receives a dressing of dung every other season. The remarkably high percentage of white clover present, in spite of mowing, was no doubt largely due to the good treatment received, and also to the fact that the hay crop is always removed early in the season, thus allowing this plant to spread a good deal in the aftermath. Ryegrass with white clover formed 75 per cent. of the herbage.

No. 11 is a meadow of above the average quality. It has been grazed by sheep, but otherwise unmanured. Compared with No. 2 its herbage shows the following points of difference:—a much smaller percentage of white clover; a much larger quantity of fiorin, and increased quantities of Yorkshire fog, cocksfoot and crested dogstail.

Nos. 17 and 18 represent two types of poor meadow land of the neighbourhood. Both are unmanured.

No. 17 lies rather dry and is grazed by sheep. The figures show that 50 per cent. of its surface was occupied by fiorin (chiefly *Agrostis vulgaris*) and other weeds.

No. 18 was grazed by horses. It suffered from want of drainage and grew herbage of which nearly 60 per cent. was Yorkshire fog, fiorin and tufted hair grass (*Aira cæspitosa*), all typical of wet soils. On the two last fields ryegrass and white clover together formed less than one-fifth of the herbage.

In Table X each of the foregoing fields are grouped according to the type of pasture and the results averaged to facilitate comparison.

TABLE IX. *Botanical Composition of the Herbage of four Meadows near Slawston.*

Reference No.	2				11				17				18		
Name	"Spence's Meadow"				"Top Meadow"				"Middle Welham"				"Clock Close"		
Date of Examination	Oct. 1905	May 19th, 1906	*Aug. 7th. 1906	*Average*	Oct. 1905	May 18th, 1906	*Aug. 7th, 1906	*Average*	Oct. 1905	May 10th, 1906	*Aug. 4th, 1906	*Average*	May 16th, 1906	Aug. 4th, 1906	*Average*
No. of places examined ...	6	8	6	*7*	8	6	4	*6*	4	6	3	*4*	6	6	*6*
Lolium perenne	20·8	16·7	41·3	*26·3*	34·2	25·7	30·0	*30·0*	11·6	9·7	14·0	*11·8*	10·6	15·3	*13·0*
Dactylis glomerata	2·0	2·5	3·4	*2·6*	4·8	6·7	11·3	*7·6*	8·0	3·1	5·5	*5·5*	—	—	—
Cynosurus cristatus	10·8	2·0	2·8	*5·2*	9·3	10·0	7·0	*8·8*	7·0	7·5	3·4	*4·8*	18·0	9·0	*13·5*
Phleum pratense...........	—	—	—	—	—	—	—	—	2·3	trace	—	*·8*	—	—	—
Avena flavescens...........	2·2	3·1	2·4	*2·6*	·3	4·6	2·7	*2·5*	2·4	4·0	trace	*2·2*	—	—	—
Poa trivialis................	4·9	4·0	trace	*2·7*	1·4	1·0	trace	*·8*	·9	1·7	trace	*·9*	5·1	—	*2·5*
Anthoxanthum odoratum	—	trace	trace	*trace*	—	·8	trace	*·3*	—	2·0	2·9	*1·6*	4·0	1·3	*2·6*
Festuca ovina	1·0	trace	2·0	*1·0*	1·0	·7	2·0	*1·2*	4·5	5·1	4·0	*4·5*	3·0	1·0	*2·0*
Agrostis alba et vars.......	—	—	trace	*trace*	14·9	13·5	12·0	*13·5*	10·8	12·3	33·0	*18·7*	17·5	29·0	*23·2*
Holcus lanatus	6·0	trace	2·4	*2·8*	8·9	3·7	8·7	*7·1*	—	1·4	1·1	*·8*	17·0	15·6	*16·3*
Aira cæspitosa	—	—	—	—	—	—	trace	*trace*	—	—	—	—	17·5	16·7	*17·1*
Alopecurus pratensis	—	—	—	—	1·0	—	—	*·3*	·7	trace	—	*·3*	3·7	trace	*1·9*
Hordeum pratense	—	—	—	—	·9	—	—	*·3*	—	—	—	—	—	—	—
Trifolium pratense	1·6	1·9	4·9	*2·8*	—	—	—	—	1·0	—	—	*·3*	—	trace	*trace*
Trifolium repens............	38·8	61·2	31·4	*43·8*	16·4	19·3	19·5	*18·4*	12·5	11·0	1·6	*8·4*	1·5	7·0	*4·2*
Lotus corniculatus	—	—	—	—	—	—	—	—	1·2	3·8	—	*1·7*	—	—	—
Achillea Millefolium	—	—	—	—	—	—	—	—	6·0	trace	trace	*2·1*	—	—	—
Ranunculus sps.	3·8	†	†	†	3·0	9·0	—	*4·0*					2·1	2·3	*2·2*
Bellis perennis	3·6	†	†	†	—	—	—	—					—	—	—
Taraxacum officinale	1·4	†	4·9	†	—	—	—	—					—	—	—
Plantago lanceolata	—	†	—	*trace*	—	—	—	—	30·0	36·9	25·7	*30·9*	—	—	—
Rumex Acetosa.............	—	—	—	—	—	2·3	—	*·8*					—	—	—
Mosses	2·7	—	1·2	*1·3*	—	1·0	trace	*·3*					—	—	—
Various weeds	—	—	—	—	·6	·7	—	*·4*					—	trace	*trace*
Bare surface.................	—	—	1·2	*·4*	—	·7	trace	*·2*	—	·8	trace	*·3*	—	2·2	*1·1*
Totals	99·6	99·4	98·8	*99·3*	96·7	99·7	93·2	*96·5*	98·9	99·3	91·2	*95·6*	100·0	99·4	*99·6*
Gramineae	47·7	28·3	54·3	*43·4*	76·7	66·7	73·7	*72·4*	48·2	46·8	63·9	*51·9*	96·4	87·9	*92·1*
Leguminosae	40·4	63·1	36·3	*46·6*	16·4	19·3	19·5	*18·4*	14·7	14·8	1·6	*10·4*	1·5	7·0	*4·2*
Other useful Plants.........	—	—	—	—	—	—	—	—	6·0	trace	trace	*2·0*	—	—	—
Weeds	11·5	8·0	7·0	*8·9*	3·6	13·0	trace	*5·5*	30·0	36·9	25·7	*30·9*	2·1	2·3	*2·2*

* Composition of aftermath.
† Indicates that the quantities of these species present were not determined separately, but have been included in the totals.

TABLE X. *Average Botanical Composition of Herbage.*

Examined twice during the summer of 1906. (1) May—June. (2) July—August.†

	Excellent Old Pastures				Excellent Recent Pastures					Good Old Pastures				Inferior Old Pastures			Meadows			
Reference No.	1	3	4		5	7	6	8		9	12	13		15	19		2	11	17	18
Name	"The Seeds"	"Cow Close"	"Hall Close"	*Average*	"The Old Churchyard"	"Watson's Seeds"	"Round Pond Field"	"Church Field"	*Average*	"High Field"	"The Big Field"	"Washpit" (Section C)	*Average*	"Solstaceway"	"Road Part"	*Average*	"Spence's Meadow"	"Top Meadow"	"Middle Welham"	"Clock Close"
No. of places examined ...	9	9	7	8	9	9	10	9	9	9	8	6	8	4	7	5	7	5	5	6
Lolium perenne	36·3	30·7	30·6	32·5	31·8	37·6	33·9	36·0	34·8	46·1	33·1	27·6	35·6	18·0	14·1	16·0	29·0	27·8	11·8	13·0
Dactylis glomerata	—	·4	1·4	·6	4·4	9·5	1·9	1·0	4·2	2·0	—	2·4	1·4	·9	9·5	5·2	2·8	9·0	4·3	—
Cynosurus cristatus	3·5	10·9	4·4	6·3	8·0	7·0	12·3	9·8	9·3	5·1	15·5	8·5	9·7	12·6	7·0	9·8	2·4	8·5	5·5	13·5
Alopecurus pratensis	·6	·5	—	·4	—	trace	trace	trace	trace	trace	trace	trace	trace	·6	—	—	—	—	trace	1·9
Phleum pratense	·9	1·0	2·7	1·5	1·0	trace	1·2	2·0	1·0	2·6	1·3	1·6	1·8	·6	—	—	—	—	trace	—
Avena flavescens	—	—	—	—	trace	·9	trace	·4	·3	1·4	—	3·3	1·6	2·5	·8	1·7	2·7	3·6	2·2	—
Poa trivialis	3·0	3·0	3·1	3·0	6·3	7·2	6·6	7·9	7·0	5·8	2·9	1·9	2·8	trace	trace	trace	2·2	·6	1·0	2·5
Poa pratensis	—	—	—	—	trace	trace	—	·3	trace	—	—	—	—	—	—	—	—	—	—	—
Poa annua	12·3	—	·9	—	4·4	2·1	2·8	1·2	2·6	·3	2·2	—	·9	—	—	—	—	—	—	—
Anthoxanthum odoratum	—	trace	—	—	—	—	—	—	—	—	3·0	—	—	1·7	1·0	1·4	trace	·5	2·5	2·6
Festuca pratensis	—	—	—	—	1·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Festuca ovina et vars.	—	trace	1·0	—	—	·8	·4	1·0	·6	·9	trace	7·5	2·8	2·3	3·4	2·9	1·1	1·4	4·5	2·0
Agrostis alba et vars.	4·2	4·5	9·5	6·1	trace	trace	3·7	3·2	1·7	6·5	4·6	4·1	5·1	37·2	18·5	27·8	trace	12·7	22·6	23·2
Holcus lanatus	1·2	2·2	5·7	3·0	·6	3·1	2·4	4·4	2·6	·7	9·0	2·6	4·1	1·3	1·1	1·2	1·3	6·2	1·3	16·3
Agropyrum repens	—	—	—	—	trace	trace	—	trace	trace	—	—	—	—	—	·6	—	—	—	trace	—
Aira cæspitosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17·1
Other grasses	—	—	—	—	—	trace	—	—	—	—	—	trace	—	—	—	—	—	trace	—	—
Trifolium pratense	—	trace	—	—	—	—	—	—	—	—	—	—	—	trace	·8	—	3·4	—	—	trace
Trifolium repens	37·2	43·6	38·6	39·8	38·6	24·4	34·0	30·8	31·9	24·0	26·0	23·8	24·6	11·6	5·1	8·4	46·3	19·4	6·3	4·2
Lotus corniculatus	—	—	—	—	—	—	—	—	—	—	—	—	—	·4	6·6	3·5	—	—	1·9	—
Achillea Millefolium	—	—	trace	—	trace	—	—	—	—	trace	—	·9	—	—	3·8	—	—	—	trace	—
Ranunculus sps.	·8	1·8	1·9	1·5	*	*	*	·5	—	3·1	1·0	2·8	2·3	5·1	4·5	4·8	*	4·5	*	2·2
Other weeds	trace	·5	trace	trace	·6	6·1	trace	1·4	1·8	·5	·6	11·3	4·1	4·1	19·1	11·6	7·5	2·1	31·3	trace
Bare surface	—	—	—	—	trace	·4	·3	—	·2	·5	trace	·8	·4	—	1·3	—	·6	·4	·5	1·1
Totals	100·0	99·1	99·8	—	96·7	99·1	99·5	99·9	—	99·5	99·4	99·1	—	98·9	97·2	—	99·3	96·7	95·7	99·6
Gramineae	62·0	53·2	59·3	58·2	57·5	68·2	65·2	67·2	64·5	71·4	71·6	59·5	67·5	77·7	56·0	66·9	41·5	70·3	55·7	92·1
Leguminosae	37·2	43·6	38·6	39·8	38·6	24·4	34·0	30·8	32·0	24·0	26·0	23·8	24·6	12·0	12·5	12·3	49·7	19·4	8·2	4·2
Other useful Plants	—	—	trace	—	trace	—	—	—	—	trace	—	·9	—	—	3·8	—	—	—	trace	—
Weeds	·8	2·3	1·9	1·6	·6	6·1	trace	1·9	2·1	3·6	1·8	14·1	6·5	9·2	23·6	16·4	7·5	6·6	31·3	2·2

NOTE. An asterisk shows that the quantity of the species was not ascertained separately, but that it has been included in the totals at the foot of the table.

† For exact dates see previous tables.

TABLE XI.

Change in Botanical Composition of Herbage caused by dry weather.

"Washpit" A, High-lying and dry. "Washpit" C, Low and moist. "Hall Close" A, Best section, dry. "Hall Close" B, Low-lying damp section.

Reference No.	13				4			
Name	"Washpit"				"Hall Close"			
Section	A		C		A		B	
Date of Examination	June 7th	July 28th	June 7th	July 28th	May 12th	Aug. 4th	May 12th	Aug. 6th
No. of places examined...	4	6	6	6	6	8	2	4
Lolium perenne	49·8	73·4	19·3	36·2	24·2	37·0	18·7	27·6
Dactylis glomerata.........	5·0	3·1	3·2	1·6	1·7	1·0	—	—
Cynosurus cristatus........	8·2	·7	13·3	3·7	7·1	1·7	5·5	14·1
Alopecurus pratensis	—	trace	—	trace	—	—	—	—
Phleum pratense............	3·7	2·2	1·9	1·4	3·4	2·0	2·0	2·3
Avena flavescens............	1·4	trace	4·5	2·2	—	—	—	—
Poa trivialis..................	3·5	·7	2·8	1·0	4·9	1·3	5·6	1·8
Poa annua.....................	·6	·6	—	—	1·8	trace	—	—
Festuca ovina	trace	trace	7·8	7·2	1·2	·7	·3	trace
Agrostis alba, var. stolonifera	2·3	8·5	1·7	6·6	7·6	11·3	12·0	13·0
Holcus lanatus..............	—	—	2·8	2·3	5·0	6·5	9·7	6·1
Trifolium repens............	23·0	4·3	18·9	28·8	40·3	36·6	23·7	29·9
Achillea Millefolium	1·0	5·1	1·9	trace	—	trace	—	—
Ranunculus Ficaria	—	—	—	—	1·4	—	19·5	—
Ranunculus bulbosus......	trace	—	5·5	trace	1·1	1·1	·3	trace
Bellis perennis	—	—	9·5	1·1	—	—	—	—
Mosses	—	trace	—	trace	—	—	—	—
Various weeds	1·3	—	6·8	5·3	trace	trace	2·7	3·4
Bare surface..................	—	·7	—	1·7	—	—	—	—
Totals	99·8	99·3	99·9	99·1	99·7	99·2	100·0	98·2
Gramineae	74·5	89·2	57·3	62·2	56·9	61·5	53·8	64·9
Leguminosae	23·0	4·3	18·9	28·8	40·3	36·6	23·7	29·9
Other useful Plants.........	1·0	5·1	1·9	trace	—	trace	—	—
Weeds	1·3	trace	21·8	6·4	2·5	1·1	22·5	3·4

NOTE. The effect of dry weather on white clover growing on dry soil is strikingly shown. The value of Yarrow as a drought-resisting plant is also indicated.

Variation of Botanical Composition of the Herbage during the Season.

Taking the average of ten fields which apparently were not affected to any great degree by their position or other circumstances, we find that during the months of May, June and July there was an increase in ryegrass and white clover to the extent of about 5 per cent. of the total herbage in each case. With weeds, crested dogstail and rough-stalked meadow grass there was a uniform decrease. In several instances the latter plant had almost disappeared by August.

The effect of drought is clearly seen in Table XI, which shows that on "Washpit" while white clover almost completely died away on the drier part, it increased considerably on the moister section.

Chemical Composition of the Herbage.

A study of Table XII shows that this varied very much with each type of pasture. For instance, in the herbage from "The Seeds" in May there was 1·73 per cent. more nitrogen and nearly twice as much phosphate as in the herbage from "Road Part" at the same date.

The analyses also indicate that some fields reached their best condition, as regards the quality of their herbage, earlier in the season than others. Thus herbage from "The Seeds," "Hall Close," and "Middle Welham" was richest in phosphate and nitrogen in early May, while the herbage of "Cow Close" and "Clock Close" appeared to be at its best three or four weeks later. Between the first week in June and the first week in August, however, there was in every instance a large increase in the dry matter, accompanied by a decrease in the percentages of nitrogen and phosphate.

Relation between the Botanical and Chemical Composition of the Herbage.

If these two series of analyses are compared it will be seen that the percentages of nitrogen and phosphate in the herbage appear to vary with the quantities of white clover and ryegrass present, especially with the former.

Nitrogen was determined in samples of each separate species from an old pasture (No. 10) in June. The percentages were: white clover 3·81, yarrow (*Achillea Millefolium*) 3·22, grasses (75 per cent. ryegrass)

TABLE XII. *Chemical Composition of Herbage and Soils of Pastures and Meadows in Leicester and Northamptonshire.*

Reference No.	Name	HERBAGE									SOIL											
	1906	Early Season May 8th			Middle Season May 28th—June 4th			Late Season August 1st—9th														
		Dry Matter	Nitrogen	P_2O_5	Dry Matter	Nitrogen	P_2O_5	Dry Matter	Nitrogen	P_2O_5	Date of Sampling	Depth of Sample, ins.	Moisture	Organic Matter and loss on ignition	Nitrogen	P_2O_5 Total	P_2O_5 Available	K_2O Total	K_2O Available	$CaCO_3$ Available	Insol. Residue
		%	%	%	%	%	%	%	%	%			%	%	%	%	%	%	%	%	%
1	"The Seeds"	19·4	4·45	1·070	21·2	3·60	1·025	38·0	2·73	·894	Oct. '05	8	3·94	11·88	·330	·798	·122	·952	·022	·340	62·10
3	"Cow Close"	20·4	3·88	·922	20·5	3·93	1·087	33·1	3·27	·775	,,	6	4·10	11·47	·366	·256	·023	·747	·011	·200	70·16
4	"Hall Close"	20·7	3·89	·900	21·5	3·52	·810	30·7	3·21	·805	,,	6	7·92	18·12	·577	·269	·019	·952	·010	·506	53·41
9	"Highfield"..	—	—	—	23·5	3·18	·900	49·6	2·04	·635	May '06	6	3·49	11·82	·358	·236	·018	·770	·026	·550	72·47
12	The "Big Field"........	—	—	—	23·0	3·18	·885	39·1	2·70	·841	,,	6	4·27	10·93	·356	·323	·021	·724	·011	·100	68·02
13	"Washpit," Sec. A......	—	—	—	18·2	3·47	·942	53·2	2·24	·645	,,	6	4·03	12·78	·418	·293	·028	·948	·042	·521	68·38
13	"Washpit," Sec. C	—	—	—	21·5	2·56	·757	57·6	1·97	·645	,,	6	4·18	12·95	·414	·312	·020	·670	·014	4·285	67·70
19	"Road Part"	28·0	2·72	·552	27·4	2·52	·742	56·0	1·87	·377	,,	5	4·61	14·42	·393	·162	·006	1·095	·030	·215	63·03
15	"Solstaceway"	—	—	—	25·1	2·34	·537	58·5	1·54	·347	Oct. '05	6	—	—	—	·180	—	·906	—	—	66·21
2	"Spence's Meadow" ...	—	—	—	17·1	3·08	·888	* 45·9	2·78	·817	—	—	—	—	—	—	—	—	—	—	—
18	"Clock Close"...........	30·0	2·26	·595	21·6	2·33	·690	47·8	1·70	·555	May '06	6	9·25	20·27	·574	·274	·022	1·100	·016	·250	45·17
11	"Top Meadow"	—	—	—	20·0	2·61	·752	* 42·6	2·16	·605	—	—	—	—	—	—	—	—	—	—	—
17	"Middle Welham"......	22·6	3·22	·670	22·0	2·85	·592	* 37·3	1·87	·392	—	—	—	—	—	—	—	—	—	—	—
7	"Watson's Seeds"	—	—	—	23·0	3·58	·988	48·0	2·12	·662	—	—	—	—	—	—	—	—	—	—	—
5	The "Old Churchyard"	—	—	—	18·1	3·77	1·003	51·7	2·82	·790	—	—	—	—	—	—	—	—	—	—	—
6	"Round Pond Field" ..	—	—	—	22·0	3·55	·901	42·0	2·75	·925	,,	6	3·49	11·20	·310	·273	·020	·852	·010	·450	67·84
8	"Church Field"	—	—	—	21·9	3·26	·884	39·5	2·71	·864	,,	6	4·94	13·00	·376	·260	·024	1·051	·028	·400	63·25
20	Cransley, Plot 6	—	—	—	†34·5	1·62	—	—	—	—	—	—	3·90	11·50	·295	·133	·013	·540	·008	—	68·30

* Aftermath. † June 21st.

2·53, and miscellaneous plants 2·14. These analyses indicate that white clover is probably the chief factor in determining the quantity of nitrogen in the herbage of a pasture.

RELATION BETWEEN SOIL AND HERBAGE.

On comparing the analyses of the soils with the chemical composition of the herbage there appears to be some relation between the quantity of available phosphates in the former and the percentages of nitrogen and phosphate in the latter. The soil of "The Seeds" contained an exceptionally high percentage of phosphates and its herbage was also very rich in phosphate and nitrogen. The herbage of this field and also of Nos. 3, 6, 8 and 13 sect. A, which are rich in phosphates, should be compared in this respect with the herbage of "Road Part" and "Solstaceway," which are comparatively poor in phosphates. Although chemical analysis shows that "Clock Close" is well supplied with phosphates, its herbage was shown by both botanical and chemical analysis to be of very poor quality. Poorness of the herbage here was evidently due to the bad mechanical condition of the soil, which made it unsuitable for the growth of the better pasture plants.

DENSITY OF HERBAGE.

It is of course evident that the value of a pasture depends not only upon the quality of its herbage but also upon the quantity produced in a season. This will depend to a considerable extent upon the density of the turf, *i.e.* the closeness of growth of the plants. By cutting away the edible herbage from a measured area in a number of places and weighing, the density of the herbage on each type of pasture could be roughly compared.

The results showed that with herbage about one inch in length the weight per square foot varied from 50 grams on a new pasture with a very open turf (Field 19, Univ. Farm), and 75 grams on types *b* and *c* (page 292), up to 85 or 95 grams on the densest turfs, *e.g.* Nos. 1 and 4.

These figures roughly indicate a large difference in the weight of herbage available per acre for grazing on each type of pasture.

TABLE XIII. *Approximate number of Plants on different types of Pastures.*

The figures show thousands of plants per acre.

Reference No.	1	3	9	12	17	19	20	8	6	14	New Pastures, University Farm			
Name	"The Seeds"	"Cow Close"	"High-field"	"The Big Field"	"Middle Wel-ham"	"Road Part"	Cransley Plot 6	"Church Field"	"Round Pond Field"	"Un-win's Pasture"	Field 19	Field 16, Elliots's Mixture	Field 16	Field 18, Un-manured
Date of Counting	May '06	May '06	May '06	June '06	May '06	May '06	June '06	June '06	June '06	Oct. '05	Sept. '05	Sept. '05	Apr. '06	June '06
No. of turfs examined	2*	2*	2*	2*	3†	2†	2*	2*	2*	1*	6†	6†	4†	4†
Lolium perenne	1730	3306	1783	1435	739	739	130	2000	1262	2740	1742	1546	1957	1044
Dactylis glomerata	—	—	478	—	348	217	391	43	—	585	319	261	261	174
Cynosurus cristatus	344	1131	565	1174	1218	1300	3262	1044	783	—	7	188	130	87
Alopecurus pratensis	43	87	—	—	87	—	—	—	—	261	14	14	—	43
Phleum pratense	215	348	609	348	43	—	—	739	—	—	94	72	87	130
Avena flavescens	—	—	348	—	565	304	435	—	—	—	—	21	87	43
Poa trivialis	1807	1522	3436	2132	174	68	—	5829	3915	1566	14	58	—	43
Poa pratensis	—	—	—	—	—	—	43	—	—	—	—	14	—	—
Poa annua	3828	—	—	217	—	—	—	87	174	—	—	—	43	—
Festuca elatior var. pratensis	—	—	—	—	—	—	—	—	—	—	130	225	87	130
Festuca ovina et vars.	—	130	—	—	130	348	565	391	261	1566	79	145	174	87
Agrostis alba et vars.	522	870	3175	783	957	560*	87	870	700	—	87	29	—	174*
Holcus lanatus	—	261	—	913	87	87	478	609	740	—	—	—	—	—
Anthoxanthum odoratum	—	—	—	130	130	87	—	—	—	—	—	—	—	—
Agropyrum repens	—	—	—	—	43	43	20	—	—	—	36	14	43	830*
Hordeum pratense	—	—	—	—	—	—	—	—	—	913	—	—	—	—
Trifolium pratense	—	—	—	—	—	—	—	—	—	—	58	87	43	43
Trifolium repens	11832	12093	6177	12789	739*	174*	1740	13920	15007	174	196	130	261	1392*
Trifolium hybridum	—	—	—	—	—	—	—	—	—	—	58	7	20	—
Trifolium minus	—	—	—	—	—	—	130	—	—	—	—	—	—	—
Lotus corniculatus	—	—	—	—	43	180	—	—	—	—	—	—	—	—
Medicago lupulina	—	—	—	—	—	—	350	—	—	—	—	7	—	—
Other useful plants	—	—	—	—	—	435	—	—	—	—	21	317	43	652
Ranunculus sps.	478	478	130	304	—	783‡	—	—	43	391	—	—	—	—
Bellis perennis	—	—	—	—	—	174	1392	—	—	870	—	—	—	—
Plantago lanceolata	—	—	—	—	—	220	696	—	—	—	—	36	—	—
Luzula campestris	—	—	—	—	—	1087	—	—	—	—	—	—	—	—
Other weeds	—	43	348	130	2914	700	6525	—	43	348	28	94	43	304
Totals in Millions	20·8	20·1	17·1	20·3	8·2	7·4	16·2	25·5	22·9	9·4	2·9	3·2	3·2	5·1
Gramineae	8·5	7·6	10·4	7·1	4·5	3·7	5·4	11·6	7·8	7·6	2·6	2·6	2·9	2·8
Leguminosae	11·8	12·0	6·2	12·8	·8	·3	2·2	13·9	15·0	·2	·3	·2	·3	1·4
Other useful Plants	—	—	—	—	—	·4	—	—	—	—	—	3	—	·6
Weeds	·5	·5	·5	·4	2·9	3·0	8·6	—	·1	1·6	—	·1	—	·3

NOTES. Each turf was one square foot in area. Those marked thus * were lifted, and each separate rooting of white clover or of grasses spreading by means of "runners" was counted as one plant. Turfs marked † were not lifted, and only the principal rootings of white clover and other plants were counted except where indicated by an *. ‡ Besides approximately 1,500,000 seedling plants per acre.

NUMBER OF PLANTS PER ACRE.

In Table XIII is given the approximate number of plants per acre on several of the fields already mentioned.

There was no difficulty in counting the plants on the new pastures without lifting the turfs, but on most of the best pastures near Market Harborough the close and interwoven growth of the plants made it almost impossible to count them even when the turfs were lifted. In these latter cases, therefore, after lifting, each separate rooting of white clover and of creeping grasses, such as rough-stalked meadow grass and fiorin, was counted as one "plant."

On fields 16 and 19 (Univ. Farm), where the limits of each plant could be readily defined, the results show that on such new pastures not more than about three or four million individual plants per acre may be present. Counting every distinct rooting of a plant on the old pastures did not of course give any accurate idea of the number of individuals present. The figures obtained, however, serve for comparison and indicate the limit to the number of individuals which can exist per acre in a pasture.

In concluding, our best thanks are due to all those gentlemen who kindly allowed us to make use of their fields in doing this work; especially to Mr E. Fisher, Market Harborough, and to Mr John Berry, of Slawston, whose knowledge of the district greatly assisted in the choice of suitable fields.

SUMMARY.

The following is a brief summary of the more important points which our investigations so far appear to have given us:—

1. That white clover and ryegrass form by far the greater part of the herbage on the best grazing lands—both old and recent in the English Midlands—and that the next most abundant species on these pastures are usually crested dogstail, fiorin (*A. stolonifera*), and rough-stalked meadow grass.

2. That the herbage of the inferior types of grass land in the same district consists very largely of bent grass (*A. vulgaris*) and various weeds, while white clover and ryegrass are present in comparatively small quantities.

3. That the only other species of grasses which are occasionally abundant in these pastures are:—cocksfoot and sheep's fescue in the better fields; Yorkshire fog and tufted hair-grass in the poorer ones.

4. That the herbage of a pasture varies botanically to a considerable extent during a season, this variation being however determined very largely by soil, situation and weather.

5. That the choicest grazing land is invariably associated with soil rich in available phosphates.

6. That on soils suitable for permanent pasture, inferiority of the herbage is generally due either to (1) a deficiency of available phosphates, or (2) to their bad mechanical condition.

7. That herbage of the best grazing land may be twice as rich in nitrogen and phosphate as that of a poor pasture, and that this large difference appears to be directly determined chiefly by the proportion of white clover present, and indirectly by the percentage of available phosphates in the soil.

8. That from the early part of June onwards the percentage of nitrogen and phosphate in the herbage of a pasture gradually decreases, while the proportion of dry matter rapidly increases.

9. That the quantity of herbage available per acre for grazing depends much upon the density of the herbage; and that no plants appear to be more capable of producing a dense growth of herbage than white clover and ryegrass, providing the soil is suitable for them.

10. That the number of individual plants per acre on the best old pastures, and necessary for the production of a thick close turf, is probably very much less than is usually supposed.

OXIDATION IN SOILS, AND ITS RELATION TO PRODUCTIVENESS.

PART II. THE INFLUENCE OF PARTIAL STERILISATION.

BY FRANCIS V. DARBISHIRE, B.A. (Oxon.), PH.D. (Leipsic) (*South Eastern Agricultural College, Wye*), AND EDWARD J. RUSSELL, D.Sc. (Lond.) (*Rothamsted Experiment Station*).

IT was shown in an earlier communication[1] that the absorption of oxygen by soil is mainly brought about by the action of micro-organisms, and is greatly diminished if the soil has previously been heated to 120° C. On one occasion the temperature of the steriliser only rose to 95°, and it was found that the rate of oxidation of the soil, instead of being reduced, was considerably increased. This unexpected result led to experiments with other methods of partial sterilisation, such as exposure to vapours of toluene, chloroform, etc., and in each case the same effect was produced, the amount of oxygen absorbed showed a marked increase after the antiseptic was removed.

Reasons were adduced in the previous paper for supposing that the rate of oxidation affords a measure of the activity of the soil micro-organisms. If this supposition is well founded we are forced to conclude from the more rapid oxidation that partially sterilising a soil so increases the activity of the surviving organisms that they use up more oxygen, and presumably bring about more decomposition, than the original organisms.

The composition of the crop affords clear evidence that the availability of the plant food in the soil has been increased by partial sterilisation. In the case of heated soils this might well have been brought about by chemical decomposition, but where volatile antiseptics were used it is difficult to see what purely chemical change can have taken place; the simplest view, and the one best agreeing with all our observations, is that the increased availability is connected with the changed bacterial flora.

[1] This *Journal*, Vol. I. 1905, p. 260.

Partial sterilisation brings about a gain in crop, and there can be little doubt that this is mainly, if not entirely, due to the extra availability of the plant food.

Our experiments show that it is possible to increase, for a time at any rate, the amount of food the plant gets from the soil.

These experiments were made at the South Eastern Agricultural College, Wye.

Effect of partial sterilisation on the rate of oxidation.

A. Experiments with volatile antiseptics.

Ten gram lots of soils were exposed for a time (generally three days) to vapours of the antiseptic, then transferred to tubes plugged with cotton-wool through which a current of washed and filtered air was drawn for 24 hours. This treatment appears to remove all the antiseptic; we were unable to detect any by smell, or, in the case of carbon disulphide, by the very sensitive triethylphosphine test. The control soil was put through precisely the same process, excepting that air was substituted for antiseptic vapour. It is very important in these experiments to make all the conditions as nearly alike as is possible. Finally the soil was put into the oxidation apparatus already described[1] and moistened with 20 per cent. of water. The amount of oxygen taken up is indicated by the rise of mercury in the gauge tube. As the experiments are only intended to be comparative it is not necessary to determine the absolute amount absorbed; for our present purpose it suffices to express the results in arbitrary units.

The soils were all from well-manured plots, one was a sand, two others were loams, and the fourth contained so much calcium carbonate that its rate of oxidation was abnormally high. Certain analytical data for the four soils are given below.

TABLE I.

Percentages calculated on soil dried at 100°.

		Loss on ignition	N	$CaCO_3$
Soil 1.	Sandy soil	3·37	·135	·39
Soil 2.	Loam, College hop garden	3·94	·170	10·7
Soil 3.	Loam, garden	6·19	·236	8·9
Soil 4.	Chalky soil, College farm		·140	59·3

The rates of oxidation are given in Table II.

[1] This *Journal*, Vol. I. 1905, p. 262.

TABLE II.

Rates of oxidation of untreated and of partially sterilised soils.

	mm. of oxygen absorbed in			Relative amounts, untreated = 100		
Soil 1. Sandy soil.	3 days	6 days	9 days	3 days	6 days	9 days
Untreated soil	3·1	7·2	11·3	100	100	100
Soil treated with carbon disulphide	9·3	17·8	20·8	300	247	184
,, ,, toluene	7·7	15·0	18·7	248	208	165
,, ,, chloroform	5·0	13·3		161	184	
Soil 2. College hop garden. Experiment 1.						
Untreated soil	2·7	5·5	6·0	100	100	100
Soil treated with carbon disulphide	7·2	9·9	11·8	267	181	197
,, ,, toluene	8·7	12·2	15·0	322	222	250
,, ,, chloroform	7·8	11·6	13·0	289	212	218
Experiment 2. Another sample of the same soil.						
Untreated soil	13·0	21·5	27·7	100	100	100
Soil treated with carbon disulphide	17·0	21·3	26·4	131	99	95
,, ,, toluene	14·1	22·3	29·2	110	104	105
,, ,, chloroform	17·3	23·5	30·8	133	110	111
Soil 3. Garden soil.						
Untreated soil	9·4	12·3	13·8	100	100	100
Soil treated with carbon disulphide	23·5	27·9	28·3	250	227	205
,, ,, toluene	12·8	24·7	27·1	136	201	196
,, ,, chloroform	10·1	15·3	16·0	108	125	116
Soil 4. Chalky soil.						
Untreated soil	5·2	8·6	13·1	100	100	100
Soil treated with carbon disulphide	5·2	11·2	19·7	100	130	151
,, ,, toluene	5·4	12·5	20·6	104	145	157
,, ,, chloroform	10·2	12·3	15·4	196	143	118
Experiment 3. Another sample of the same soil.						
Untreated soil	3·9	5·9	8·0	100	100	100
Soil treated with carbon disulphide	17·1	21·4	23·9	439	363	299
,, ,, toluene	2·0	8·5	12·2	52	144	153
,, ,, chloroform	1·3	4·8	6·3	33	81	79

The readings for the duplicate untreated soils agree to within about 1 mm.

Each soil was made the subject of several experiments. Two sets of results are given for soils 2 and 4: they are fairly typical of the rest. They are not directly comparable, because they were not obtained at the same time or under the same temperature conditions, but they show clearly that the figures must be interpreted in a qualitative way only and have no quantitative significance, a fact which is also demonstrated by the variation in the relative rates from day to day. Sometimes one antiseptic and sometimes another has been found most potent, occasionally no difference in the rate has been produced, and it has happened that the rate has been depressed by the treatment. But

taking the results as a whole it is quite evident that the rate of oxidation has been increased by partial sterilisation.

Apparently it is not essential to draw a current of air over the soil for 24 hours, though we always considered it desirable to do so. In one experiment the soil after removal from the antiseptic vapour was exposed for three minutes only to air and then transferred to the oxidation apparatus. It is practically certain that some trace of the antiseptic must have remained, yet the rate of oxidation increased considerably.

The results are given in Table III.

TABLE III.

Rates of oxidation when traces of the antiseptic were probably present.

	mm. of oxygen absorbed in			Relative amounts, untreated = 100		
	3 days	6 days	8 days	3 days	6 days	8 days
Untreated soil	3·4	2·9	4·1	100	100	100
Soil treated with carbon disulphide	6·8	6·3	8·7	200	217	212
,, ,, toluene	6·4	7·3	11·5	188	252	280
,, ,, chloroform	11·1	11·6	14·5	326	400	354

It does not appear to be possible to sterilise a soil completely by treatment with any of these antiseptics.

Some experiments have also been made with formalin. This differs from the other antiseptics because it cannot be completely removed from the soil; apparently polymerisation or some combination with a soil constituent takes place. The rate of oxidation is very considerably reduced, often indeed to zero.

B. Experiments with non-volatile antiseptics.

Ten grams of soil were mixed with small quantities of various poisons either by grinding in a mortar or by adding solutions of the proper strength. There are difficulties about either method. Grinding increases the rate of oxidation, probably by effecting a better distribution of the organisms or their food-stuffs, and it is hardly possible to ensure the same amount of grinding for all the soils. On the other hand, it is quite impossible to secure uniform distribution of a dissolved body in the soil owing to the property soils have of withdrawing substances from their solutions. We have usually found that grinding gives more even results.

Copper sulphate and mercuric chloride have been used in various strengths and the results are given in Table IV. It will be observed that very small amounts have no effect, while larger quantities check oxida-

tion. In some experiments there is evidence of a slight stimulating effect when about ·01 per cent. of poison is present, but this is not always the case, and we have not been able to discover the conditions under which the stimulus shows itself.

The amount of poison which may be present in the soil without retarding oxidation is very remarkable; even 0·1 per cent. of mercuric chloride does not inhibit it and yet it would effectually sterilise a solution. The explanation appears to be that the poison does not get really into the soil in spite of the grinding and the subsequent addition of 20 per cent. of water, and hence many of the organisms escape.

TABLE IV.

Rates of oxidation of soils treated with non-volatile poisons.

Copper sulphate and hop garden soil.

Amount of copper sulphate	mm. of oxygen absorbed in 3 days	6 days	9 days	Relative amounts, untreated = 100 3 days	6 days	9 days
None	6·7	12·7	15·1	100	100	100
·01 per cent.	6·3	12·4	14·9	94	98	99
·1 ,,	4·5	11·0	14·4	67	87	95
1 ,,	4·1	8·4	10·5	61	66	69

Copper sulphate and gault pasture soil.

Amount of copper sulphate	mm. of oxygen absorbed in 3 days	6 days	9 days	Relative amounts, untreated = 100 3 days	6 days	9 days
None	10·3	17·5	26·6	100	100	100
·001 per cent.	12·6	20·2	28·9	122	115	109
·01 ,,	13·8	18·5	29·2	134	106	110
1 ,,	2·2	3·7	8·2	21	21	31

Mercuric chloride and fertile arable soil.

Amount of mercuric chloride	mm. of oxygen absorbed in 3 days	6 days	10 days	13 days	Relative amts. untreated = 100 6 days	10 days	13 days
None	2·9	7·3	10·3	13·6	100	100	100
·001 per cent.	3·8	7·0	9·9	15·3	96	97	123
·01 ,,	3·9	9·3	12·4	13·8	127	120	100
·1 ,,	2 1	2·0	2·8	4·3	29	27	32
1 ,,	nil	nil	nil	?	nil	nil	?

Mercuric chloride and hop garden soil.

Amount of mercuric chloride	mm. of oxygen absorbed in 5 days	8 days	11 days	14 days	Relative amts. untreated = 100 5 days	8 days	11 days	14 days
None	8·0	10·2	13·3	17·2	100	100	100	100
·001 per cent.	5·1	9·0	12·7	14·5	64	88	95	84
·01 ,,	1·7	4·4	7·0	7·8	21	43	53	57
·1 ,,	1·1	1·2	1·5	4·1	14	12	12	24

C. Experiments with heated soils.

In our first experiments the soil and the whole oxidation apparatus were heated in a steam-oven to 95° for two hours, but later on it became evident that there was no advantage gained by including the apparatus, and the soil only was heated. The results, given in Table V, show

clearly that the effects of heating to 95° is to considerably increase the rate of oxidation.

TABLE V.

Rates of oxidation of heated soils.

	mm. of oxygen absorbed in			Relative amounts		
	3 days	6 days	9 days	3 days	6 days	9 days
Hop garden soil, unheated ...	3·7	5·2	7·0	100	100	100
,, ,, heated to 95°	6·0	8·2	12·0	160	158	171
Garden soil, unheated	7·5	10·2	15·5	100	100	100
,, heated to 95°......	16·9	27·2	33·2	226	267	215

Effect of partial sterilisation on productiveness.

Earlier investigations. That carbon disulphide favourably influences plant growth has long been known. Girard[1] had used it in France to clear a piece of sugar-beet ground badly infested with nematodes, and observed marked increases in the succeeding crops; and Oberlin, an Alsatian vine-grower, who had long used carbon disulphide for killing phylloxera[2], noticed that it also increased the productiveness of the soil. These results, published independently in 1894, drew general attention to the subject, the experiments were repeated, confirmed and extended, and a number of papers have since appeared. From the practical side Behrens[3] showed that application of carbon disulphide doubled the crop on "onion sick" ground; Mach[4] found that 200 grams per square metre augmented the yield of oats, potatoes and beets, and Henry[5], using double this quantity, obtained large increases in the growth of acacias and other plants.

Pot experiments were made by Pagnoul[6], and also by Koch[7] who found that the effect increased up to a certain point with increasing amounts of carbon disulphide:—

CS_2 per pot, c.c.	0	25	60	100	200	300
Yield of mustard, grams	13·25	14·92	21·83	18·68	37·58	22·3
,, buckwheat, grams	36	78	94	93	99	90

[1] *Bulletin de la Société Nationale d'Agriculture en France*, 1894, Vol. 54, p. 356.

[2] *Bodenmüdigkeit und Schwefelkohlenstoff*, Mainz, 1894, Zabern: also *Journal d'Agriculture pratique*, 1895, 1, 459. Carbon disulphide appears to have been used as an insecticide 50 years ago in Algeria for destroying grain pests, v. Akbar, 1857, Oct. 16, quoted in *Gardeners' Chronicle*, 1858, Aug. 28, 653.

[3] Koch's *Jahresbericht über die Fortschritte in der Lehre von den Gärungsorganismen*, 1895, 6, 280.

[4] *L'Engrais*, 1896, 543.

[5] *Bul. Soc. Sci. Nancy*, 1901, 27, abs. in *Expt. Station Record*, 1902, 13, 528.

[6] *Annales Agronomiques*, 1895, 21, 497.

[7] *Arbeiten der deutschen Landwirtschafts-Gesellschaft*, Heft 40, 1899.

Wollny's[1] pot experiments also showed a gain in crop, and, more recently, Nobbe and Richter[2] have obtained similar results with ether, chloroform and benzene.

Weight of oats, dry matter in grams.

	Untreated soil	Soil treated with ether	CS_2	$CHCl_3$	C_6H_6
I.	26·16	34·26	41·39	37·13	40·78
II.	29·75	35·19	36·40	34·01	35·77

Experiments on heated soils. The earliest observations on the effect of heat on soil arose out of bacteriological investigations. It had at first been assumed that no change took place, but Franke[3] in 1888 demonstrated the incorrectness of this view and obtained larger crops of oats and of yellow lupines on heated than on unheated soil; he showed also that heating increased the solubility of the mineral and of the organic matter in the soil. Five years later, Liebscher[4], who was engaged in one of the periodical revivals of the question whether plants can assimilate free nitrogen, stated that the sterilisation of soil by steam increases the availability of the phosphates and nitrogen compounds. Later experiments have confirmed this, but do not take us much further. Pfeffer and Franke[5], working with mustard, confirmed the gain in productiveness and obtained an increased assimilation of nitrogen, which suggests that the nitrogen compounds have become more available.

	Unmanured soil		Soil + $NaNO_3$	
	Unsterilised	Sterilised	Unsterilised	Sterilised
Weight of crop	14·8	27·6	62·4	71·2
Weight of N taken up	·1546	·4323	1·1938	1·4688

Krüger and Schneidewind[6] carried out a somewhat fuller series of experiments and strikingly demonstrated the effect on the availability of the mineral matter. This soil evidently stood in no need of nitrogenous manure, but was lacking in mineral food-stuffs, and a gain

[1] *Vierteljahrsschrift d. bayerischen Landwirtschaftrats*, 1898, Heft 3, 319, abs. in *Bied. Centr.* 1900, 29, 146.

[2] *Landwirtschaftlichen Versuchs-Stationen*, 1904, 60, 433.

[3] *Berichte der Deutschen botanischen Gesellschaft*, 1888, 89, 6 (Generalversammlungs Heft).

[4] *Deutsche Landw. Presse*, 1893, No. 94, 976. Liebscher and Wagner were, on this occasion, the two protagonists, and discussion continued for some time in the *Deutsche Landw. Presse.*

[5] *Landw. Versuchs-Stat.* 1896, 46, 117.

[6] *Landw. Jahrbücher*, 1899, 28, 224.

in crop was produced when these were either added or set free by heat.

	Weight of mustard			
	No manure	$NaNO_3$	Complete mineral manure	$NaNO_3$ + complete mineral manure
Untreated soil ...	17·3	17·5	33·7	50·9
Sterilised soil ...	33·2	36·5	46·9	62·4

Dietrich[1] also obtained increased productiveness when working with arable and garden, but not with pasture soils. He concluded that when the soils are heated there arises some poisonous substance to which mustard is very sensitive, whilst oats, buckwheat, and peas are much less so. An amplified account was given by C. Schulze in 1906[2].

Koch and Lüken[3] obtained very similar results with oats grown on a very poor sand containing only ·016 per cent. of nitrogen. Equal amounts of nitrate of soda were supplied to the sterilised and to the unsterilised pots, but the plant got more nitrogen from the former than from the latter. The authors suggest that nearly 10 per cent. of the nitrogen of the sterilised sand was taken up.

Practical horticulturalists have for some time used steam for killing insect pests, nematodes, etc., in greenhouse soils, and there are several records of gains in the rate of plant growth. Stone and Smith of the Hatch Experiment Station, Massachusetts, dealt with the subject in 1898[4] and at a later date[5] recommended heating greenhouse soil for the sake of the extra crop obtained.

Our experiments. We have made a number of pot experiments, usually with soil 2 (College hop garden), in the glass-house attached to the laboratory. One or two hundredweights of soil which had passed a half-inch sieve was carefully turned half a dozen times in the manner adopted by the horticulturalist for mixing potting soils, and at the same time all earthworms, insects, etc., were picked out. The pots used were Doulton's glazed stoneware jars of two gallons capacity, technically known as mixing pans, they were filled with equal weights of soil (usually 16 kilos), and samples were drawn from each pot for determination of the amount of nitrate and of water present. This gives a ready means of ascertaining whether the soil is uniform in composition, though

[1] *Jahresbericht der landw. Versuchs.* Marburg, 1901-2, p. 16, abs. in *Bied. Centr.* 1903, 32, 68.

[2] *Landw. Versuchs-Stat.* 1906, 65, 137.

[3] *Jour. für Landw.* 1907, 55, 161.

[4] *Mass. Hatch Station Bul.* 55, see *Expt. Station Record*, 1899, 10, 1055.

[5] *Report of Hatch Station*, Mass., 1903, p. 38.

of course it does not indicate whether the packing is the same in all pots. The soil was then partially sterilised by one of the methods described below.

No manure of any sort was added.

After treatment, and before sowing, the pots in each series were brought to the same degree of moistness by adding the proper quantity of water, and they were all kept equally moist during the experiment. Sterilised tap-water was used throughout.

TABLE VI.

Weight and composition of crops grown in soils treated with volatile antiseptics.

(*a*) *Buckwheat* (April 9th to June 19th, 1907)

	Dry matter of crop, gms.	Relative weights of dry matter	Composition of dry matter N per cent.	P_2O_5 per cent.	K_2O per cent.
Untreated soil	18·14	100	2·75	1·87	5·62
Soil treated with $CHCl_3$ ...	25·08	138	2·91	2·45	5·65
,, ,, CS_2	23·27	128	3·15	2·34	5·97
,, ,, toluene..	20·98	116	3·00	2·12	5·79

	(*b*) *Buckwheat* (April 23 to July 15, 1906) Total wt. of crop, gms.	Dry matter of crop, gms.	Relative weights of dry matter	(*c*) *Mustard* (May 22 to July 14, 1906) Total wt. of crop, gms.	Dry matter of crop, gms.	Relative weights of dry matter
Untreated soil	80·6	15·9	100	39·88	8·00	100
Soil treated with $CHCl_3$ ...	105·2	23·0	143·8	50·4	9·9	124
,, ,, CS_2	114·3	27·2	171·1	50·96	9·48	118
,, ,, toluene...	—	—	----	43·44	8·9	111
,, ,, ether......	89·0	19·3	121·4	—	—	—
,, ,, benzene .	—	—	—	46·4	9·7	121

Details of Experiment (*a*).

No. of pot	Treatment	N in dry soil per cent.	Total weight of crop, gms.	Dry matter of crop, gms.	Composition of dry matter N per cent.	P_2O_5 per cent.	K_2O per cent.
57	untreated	·163	189·25	18·75	2·85	1·90	6·63
75	,, ...	·161	147·85*	17·53	2·65	1·83	4·61
55	chloroform	·159	228·7	27·52	3·06	2·52	5·39
56	,, ...	·156	197·50	22·65	2·76	2·38	5·91
59	carbon disulphide	·159	198·2	21·52	2·98	2·23	6·20
76	,, ,,	·161	224·5	25·02	3·33	2·56	5·75
58	toluene	·164	149·5	18·11	3·06	1·92	5·56
74	,, ...	·166	220·6	23·85	2·96	2·32	6·03

* 75 was cut some hours later than 57, and had lost water in the meantime.

We are indebted to Mr E. C. Chapelow, the College horticulturalist, for much assistance with the management of the plants.

A. Experiments with volatile antiseptics.

A definite amount (25 or 50 c.c.) of the antiseptic having been poured into the soil, the pot was covered up and left for a week. The soil was then spread in a thin layer and stirred at frequent intervals until 36 hours after all smell of the substance had passed off; this usually took about three days. The unsterilised soils were spread out and stirred in precisely the same manner.

TABLE VII.

Residual effect of volatile antiseptics.

Experiment 1.

		Untreated soil	Soil treated with $CHCl_3$
(*a*) 1*st crop, Turnips* (Aug. 29, 1905 to Feb. 18, 1906)	Fresh weight	126·3	225·0
	Dry matter	13·0	25·25
	Relative weights of dry matter	*100*	*194*
(*b*) 2*nd crop, Wheat* (Feb. 20 to July 26, 1906)	Straw	22·5	27·9
	Relative weights	*100*	*124*
	Grain	7·98	8·75
	Relative weights	*100*	*107*
(*c*) 3*rd crop, Buckwheat* (Aug. 16 to Oct. 20)	Dry matter	6·15	5·75
	Relative weights	*100*	*94*
(*d*) 4*th crop, Rye* (Dec. 21, 1906 to July 27, 1907)	Straw	49·45	32·28
	Relative weights	*100*	*65*
	Grain	16·4	19·2
	Relative weights	*100*	*117*

Experiment 2.

	(*e*) 1*st crop, Mustard* (Aug. to Oct. 1906)			(*f*) 2*nd crop, Rye* (Dec. 1906 to July, 1907)			
				Grain		Straw	
	Green weight, gms.	Dry matter, gms.	Rel. wts. of dry matter	gms.	Relative weights	gms.	Relative weights
Untreated soil	118·0	18·12	100	11·48	100	29·05	100
Soil treated with $CHCl_3$	150·5	23·15	127·7	14·46	126	40·00	135
,, ,, CS_2	117·6	19·81	109·4	15·33	133	41·04	141
,, ,, toluene	123·3	21·11	116·5	14·55	127	34·40	119

Percentage composition of dry matter of Rye, Experiment 2 (*f*)

	Grain N per cent.	Grain P_2O_5 per cent.	Straw N per cent.	Straw P_2O_5 per cent.
Untreated soil	1·15	1·041	·517	·387
Soil treated with $CHCl_3$	1·15	·939	·588	·395
,, ,, CS_2	1·28	1·094	·588	·542
,, ,, toluene	1·29	1·095	·521	·468

The results of the experiments, and the dates of sowing and harvesting, are set out in Tables VI and VII. In one instance all the

figures are given to show the magnitude of the experimental error: in other cases mean values only are given.

The duplicate untreated pots agree to within 5 or 7 per cent. but the duplicate treated pots show much larger differences—20 to 27 per cent.—and repetition of the experiment usually places the antiseptics in a different order.

The results have no precise quantitative significance but are to be interpreted qualitatively, just as was the case for the rates of oxidation. What they do show is that partial sterilisation of the soil with volatile antiseptics causes an increase in crop. Pot experiments with other plants, spinach, cabbage, etc., and field trials with certain market garden crops, give similar results.

The action extends over more than one crop: there is a distinct residual effect seen in the second, but not in the third, crop. In one experiment turnips were followed successively by wheat, buckwheat, and rye without any further treatment whatsoever; in another experiment rye was taken after mustard. The results are given in Table VII.

Experiments with formalin. Although the soil was exposed to air for some time the formalin was not entirely removed, and it had a remarkable effect on the growth of turnips, the only crop yet tried. Germination was greatly retarded, and when finally the young plants appeared they were weak and a number died. After some weeks, however, the survivors picked up very rapidly and growth continued vigorously to the end. The results were :—

	Green weight, grms.	Dry matter	Relative weights of dry matter
Untreated soil	40·4	6·4	100
Soil treated with formalin...	68·4	16·0	250

B. Experiments with non-volatile antiseptics.

Kilogram lots of the soil were thoroughly mixed in a mortar with the proper quantity of the substance under investigation. The control soil was also put through an identical stirring process. The soils were then replaced in the pots, the requisite amount of water added, and the seeds sown.

The antiseptics used have been mercuric chloride, copper sulphate and the milder poison thymol. The results are given in Table VIII., they differ fundamentally from those obtained with volatile antiseptics for in no case has any increased productiveness been observed.

We cannot explain the abnormally low result shown by buckwheat with ·001 per cent. of thymol.

Table VIII.

Crops grown in soils mixed with non-volatile antiseptics.

(*a*) *Mercuric chloride.* Buckwheat, sown Aug. 3rd, 1906, pulled Oct. 5.

Mercuric chloride added	1 per cent.	0·1 per cent.	·01 per cent.	·001 per cent.	none
Total weight of crop ...	nil	1·95	28·0	60·75	75·85
Weight of dry matter ...	nil	·33	5·55	13·25	14·7

(*b*) *Copper sulphate.*

		Untreated soil	·01 per cent. copper sulphate
Oats (Nov. 1, 1905 to July 5, 1906)	Straw...............	23·8	23·7
	Grain	11·4	13·5
Maize (Nov. 2, 1905 to July 5, 1906)	Dry matter	41·0	43·0

(*c*) *Thymol.*

		Untreated soil	·01 per cent. thymol	·001 per cent. thymol
Oats (as above)	Straw	23·8	19·3	19·8
	Grain	11·4	11·9	11·9
Maize (as above)	Dry matter......	41·0	43·0	43·0

Buckwheat, sown Aug. 27, 1906, pulled Oct. 5.

Thymol added	0·1 per cent.	·01 per cent.	·001 per cent.	none	none
Total weight of crop	11·2	28·30	22·30	31·51	29·00
Weight of dry matter ...	·8	2·82	1·43	3·42	2·90

C. Experiments with heated soils.

The pots were placed for two to three hours in a galvanised iron vessel through which a rapid current of steam was passed, giving a temperature of about 90° to 95° C. After they had cooled, they were brought to the same water content as the untreated pots. The increase in crop is very striking, as is seen by the results in Table IX.

With the exception of dwarf beans leguminous crops show no increase. No nodules form in the heated soils. Some of our experiments show that it is easier to get a second crop of a leguminous plant from a heated than from an unheated soil.

Table IX.

Crops grown in heated and in unheated soils.

(*a*) *Wheat*[1].

	Weight of crop				Composition of crop				
	Straw		Grain		Straw			Grain	
	Grms.	Rel. wts.	Grms.	Rel. wts.	N per cent.	P_2O_5 per cent.	K_2O per cent.	N per cent.	P_2O_5 per cent.
Unheated soil ...	36·23	100	10·02	100	·287	·361	1·89	1·89	·94
Heated soil	80·08	221	36·3	360	·341	·507	2·03	1·95	·95

[1] Grown in hop garden soil, containing ·161 per cent. N.

(*b*) *Various non-leguminous crops*[1].

		Unheated soil Grms.	Heated soil Grms.	Heated soil Relative weight, unheated = 100
1. Spinach,	green weight.........	10·55	47·2	449
	dry matter	1·7	6·45	379
2. Tobacco plant,	green weight.........	66·6	169·8	255
	dry matter	9·9	31·2	315
3. Tomato,	green weight.........	49·2	189·5	385
	dry matter	7·0	28·3	404
4. Verbena,	green weight.........	24·9	100·5	403
	dry matter	5·6	26·8	479
5. Turnips,	green weight.........	96·8	154·8	160
	dry matter	12·4	18·0	145
6. Lettuce,	green weight.........	128·6	170·7	133
	dry matter	10·24	17·25	168

(*c*) *Leguminous plants.*

		Unheated soil Grms.	Heated soil Grms.	Heated soil Relative weight, unheated = 100
1. Sweet peas,	green weight.........	180	173	96
	dry matter	44	34	77
2. ,, ,,	green weight.........	59·45	61·2	103
	dry matter	7·3	8·4	115
3. Dwarf beans,	green weight.........	66·0	134·4	204
	dry matter	9·0	21·6	240
4. Red clover,	green weight.........	16·6	15·8	95
	dry matter	3·35	3·1	92
5. Sainfoin	green weight.........	26·75	26·49	98
	dry matter	6·35	6·17	97

There is a distinct residual effect. Three sets of experiments have been made to test this point, and in each case the second, and in one case the third crop shows an increase.

TABLE X.

Residual effect shown by heated soils.

(*a*) Soil steamed Dec. 13th, 1906, sown with mustard Dec. 20th, this was pulled April 11th, 1907, and without further treatment Buckwheat was sown April 12th and pulled June 17th.

Weight and composition of crop

	1st crop. *Mustard*				2nd crop. *Buckwheat*			
		Composition of dry matter				Composition of dry matter		
	Dry matter, grms.	N per cent.	P_2O_5 per cent.	K_2O per cent.	Dry matter, grms.	N per cent.	P_2O_5 per cent.	K_2O per cent.
Unheated soil (mean of 2)	15·88	2·30	1·00	4·20	13·79	1·24	2·38	4·03
Heated soil (mean of 4) ...	24·33	4·43	2·08	5·02	27·40	2·00	2·26	4·74

[1] 1 to 4 were grown in arable soil containing ·110 per cent. N and losing 4·64 per cent. on ignition, whilst 5 and 6 were in garden soil containing ·138 per cent. N and losing 7·0 per cent. on ignition.

(*b*) Soil steamed August, 1905, and without further treatment 4 successive crops were raised.

		Unheated soil	Heated soil
1st crop, Turnips (Aug. 29, 1905 to Feb. 18, 1906)	Fresh weight	126·3	277·8
	Dry matter	13·0	31·25
	Relative weights of dry matter	*100*	*240*
2nd crop, Wheat (Feb. 20 to July 26)	Straw	22·5	31·87
	Relative weights	*100*	*142*
	Grain	8·0	10·73
	Relative weights	*100*	*134*
3rd crop, Buckwheat (Aug. 16 to Oct. 20)	Dry matter	6·15	5·62
	Relative weights	*100*	*91*
4th crop, Rye (Dec. 21, 1906 to July 27, 1907)	Straw	49·45	40·95
	Relative weights	*100*	*83*
	Grain	16·40	15·39
	Relative weights	*100*	*94*

(*c*) Soil steamed Jan. 1906, and cropped with *radishes*. Green weights only given.

	1st crop Feb.—April	2nd crop April—June	3rd crop June—July	4th crop July—Sept.	5th crop Sept.—Dec.	6th crop Dec.—Apr. 1907	7th crop Apr.—June
Unheated soil	22·56	18·0	22·8	29·9	7·48	20·35	30·54
Heated soil...	48·65	64·0	40·4	38·3	7·75	23·25	29·75

The soil was then heated to 95° for 2 hours and the 8th crop was sown.

Unheated soil	25·7
Heated soil	47·85

(*d*) Soil steamed Aug. 1906, two successive crops raised.

	1st crop, Buckwheat (Aug. 27th to Oct.)		*2nd crop, Rye* (Dec. 21st to July 27th, '07)			
	Green wt.		Straw		Grain	
	grms.	Rel. wt.	Grams	Rel. wt.	Grams	Rel. wt.
Unheated soil	40·56	100	68·10	100	29·39	100
Heated soil	48·05	119	95·20	140	57·11	194

Percentage composition of dry matter of the Rye crop.

	Straw		Grain	
	N per cent.	P_2O_5 per cent.	N per cent.	P_2O_5 per cent.
Unheated soil	·383	·278	1·10	·874
Heated soil	·454	·141	1·28	·888

In experiment (*a*) pasture soil was used containing ·182 per cent. N and losing 4·3 per cent. on ignition. Earlier investigators have sometimes stated that mustard suffers very much when grown in heated pasture soils; we have never observed any retardation at all, the plants do well right from the beginning to the end. All our soils, however, contain a liberal amount of calcium carbonate—somewhere about 10 per cent.

Effect of reinoculation after partial sterilisation by heat. Well water was used for watering. The heated soils were divided into two lots, one receiving sterilised, the other unsterilised water. The results, given in Table XI., are very interesting, and throw light on the whole

subject. They appear to indicate that so long as the new flora obtained after heating was left undisturbed, as it is by watering only with sterilised water, the usual increase in crop is obtained, but when the new flora is disturbed by the addition of unsterilised well water the crop decreases.

FIG. 1. Buckwheat growing in (1) unheated, (2) heated soil (see Table X.).

The ash of spinach grown on the heated soils was green, indicating the presence of manganese; that from the unheated soil was practically white.

Soils heated to higher temperatures. A few experiments have been made with soils heated to 120° C. The same kind of results are obtained as at the lower temperatures, but they are somewhat intensified. They are given in Table XII.

TABLE XI.

Effect of reinoculation.

		Heated soil, water sterilised	Heated soil, water unsterilised	Unheated soil, water sterilised
Spinach.	Dry matter	15·40	11·35	9·65
	Relative weights	*160*	*118*	*100*
	N per cent.	4·94	4·80	2·59
	Weight of N in crop	·761 gms.	·545 gms.	·250 gms.
Radishes.	Dry matter	4·50	2·17	1·75
	Relative weights	*257*	*124*	*100*
Clarkias.	Dry matter	6·80	4·86	2·86
	Relative weights	*238*	*170*	*100*

TABLE XII.

Effect of higher temperatures.

	Lettuce			Dianthus			Sainfoin		
	Green wt.	Dry matter	Rel. wt. of dry matter	Green wt.	Dry matter	Rel. wt. of dry matter	Green wt.	Dry matter	Rel. wt. of dry matter
Unheated soil ...	70·3	6·35	100	37·57	10·01	100	26·75	6·35	100
Soil heated to 80°	87·1	7·4	116·5	49·15	11·53	115	26·49	6·17	97·16
,, ,, 120°	108·5	9·4	148·0	72·06	17·54	175	19·45	4·52	71·2

Effect of partial sterilisation on the composition of the crop and on the amount of plant food removed from the soil.

Analysis of the crop shows that plants grown on sterilised soils usually contain an increased percentage of nitrogen and of phosphoric acid, notwithstanding their extra weight: indeed it would appear that there is often a wasteful consumption of these two substances, so great is the change in percentage composition. Even the second crop sometimes shows this difference.

The effect of volatile antiseptics is shown by buckwheat (Table VI. (*a*)), and by rye (Table VII. (*f*)): that of heat by mustard, buckwheat, rye (Table X. (*a*) and (*d*)) and wheat (Table IX. (*a*)).

The total amount of plant food withdrawn from sterilised soils is in consequence much greater than from the untreated soils: the quantities are given in Table XIII.

Discussion of results. The two main facts brought out in the course of our experiments are that after the soil has been partially sterilised there is an increase in the amount of oxygen absorbed in the soil, presumably by the micro-organisms, and also an increase in crop productiveness.

TABLE XIII.

Weights of food materials taken by plants from variously treated soils.

Amounts taken by Buckwheat (Table VI. (*a*))

	N Gms.	N Rel. wts.	P_2O_5 Gms.	P_2O_5 Rel. wts.	K_2O Gms.	K_2O Rel. wts.
Untreated soil	·499	100	·339	100	1·019	100
Soil treated with $CHCl_3$...	·730	146	·615	181	1·413	139
,, ,, CS_2......	·733	147	·544	161	1·389	136
,, ,, toluene.	·629	126	·445	131	1·215	119

These results are set out in diagram form in Fig. 2.

Amounts taken by Rye (Table VII. (*f*))

	N In straw, gms.	N In grain, gms.	N Total gms.	N Rel. wts.	P_2O_5 In straw, gms.	P_2O_5 In grain, gms.	P_2O_5 Total gms.	P_2O_5 Rel. wts.
Untreated soil	·075	·132	·207	100	·056	·119	·175	100
Soil treated with $CHCl_3$...	·120	·166	·286	138	·081	·136	·217	124
,, ,, CS_2......	·113	·196	·309	149	·104	·167	·271	155
,, ,, toluene.	·082	·188	·270	131	·074	·159	·233	133

Successive amounts taken from heated soils (Table X.)

Amounts taken by 1st crop (Mustard)

	N Gms.	N Relative weights	P_2O_5 Gms.	P_2O_5 Relative weights	K_2O Gms.	K_2O Relative weights
Untreated soil ...	·367	100	·159	100	·668	100
Heated soil	1·077	293	·506	318	1·221	183

Amounts taken by 2nd crop (Buckwheat)

	N Gms.	N Relative weights	P_2O_5 Gms.	P_2O_5 Relative weights	K_2O Gms.	K_2O Relative weights
Untreated soil......	·171	100	·320	100	·555	100
Heated soil	·548	320	·619	194	1·299	234

Total amounts taken by the two crops

	N Gms.	N Relative weights	P_2O_5 Gms.	P_2O_5 Relative weights	K_2O Gms.	K_2O Relative weights
Untreated soil ...	·538	100	·479	100	1·223	100
Heated soil	1·625	302	1·125	235	2·520	206

These results are set out in diagram form in Fig. 3.

Amounts removed by Wheat (Table IX. (*a*))

	N In straw, gms.	N In grain, gms.	N Total gms.	N Rel. wts.	P_2O_5 In straw, gms.	P_2O_5 In grain, gms.	P_2O_5 Total gms.	P_2O_5 Rel. wts.
Untreated soil......	·114	·227	·341	100	·089	·113	·202	100
Heated soil	·244	·708	·952	279	·224	·345	·569	282

Amounts removed by Rye (Table X. (*d*))

	N In straw, gms.	N In grain, gms.	N Total gms.	N Rel. wts.	P_2O_5 In straw, gms.	P_2O_5 In grain, gms.	P_2O_5 Total gms.	P_2O_5 Rel. wts.
Untreated soil......	·261	·323	·584	100	·085	·257	·343	100
Heated soil	·432	·731	1·163	199	·100	·507	·607	178

It is manifest that the bacterial flora of the soil must be profoundly changed by the process, but until the change has been more fully investigated it is useless to discuss the increased absorption of oxygen. Experiments on this question are now in hand.

We are also unable to state the ultimate cause of the increase in crop production.

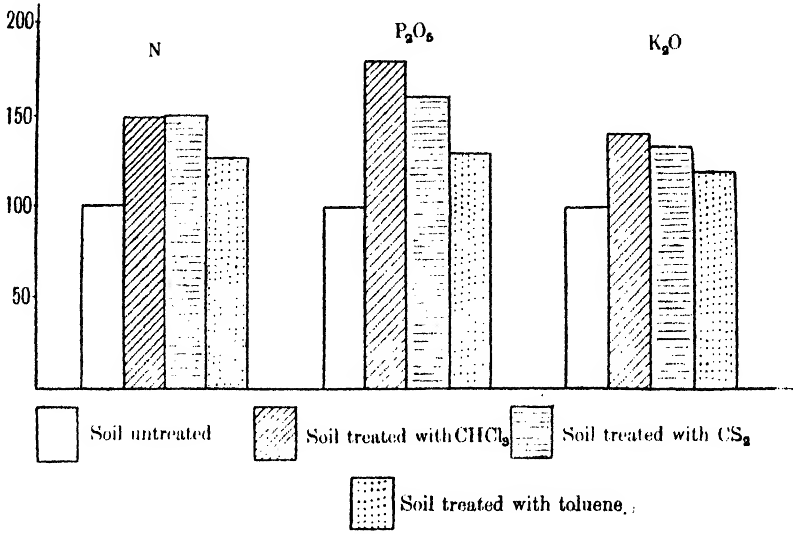


FIG. 2. Relative amounts of plant food taken by buckwheat from variously treated soils (see Tables VI. (*a*) and XIII.).

The treatment has no obvious effect on germination, the young plants appear to be all equal in size and differences only show themselves at a later date. Whatever produces the increased crop, it certainly is not an initial stimulus giving the plants such a start that the others can never overtake them; the cause persists throughout the life of the plant and even lasts on to influence a second crop. The immediate cause of the increased productiveness is no doubt the increased availability of the plant food, but how this comes about is not altogether clear.

In the case of heated soils some chemical decomposition certainly takes place. A heated soil on treatment with water yields a larger amount of soluble matter and the extract is darker in colour than before. The increase in "available" plant food is strikingly shown in Table X. (*a*) by the amounts actually removed by the plant from the

soil. From the heated soil the two crops can get three times as much nitrogen, and twice as much phosphoric acid and potash, as from the untreated, while the difference in composition of the dry matter is very considerable. The crop grown on the untreated soil is quite normal, and shows no sign of starvation or defective metabolism; its content of nitrogen and phosphoric acid seems sufficient. On the heated soils so much of these substances is presented to the plant that their percentage amount in the dry matter almost doubles; apparently we are dealing with a "luxus consumption."

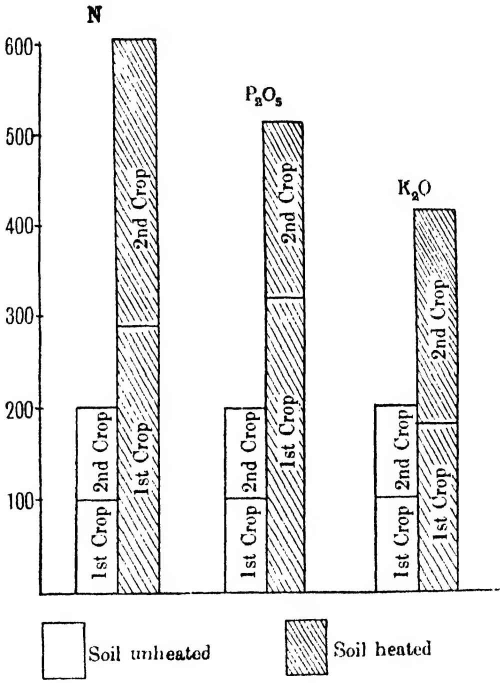


FIG. 3. Relative amounts of food taken by plants from heated and unheated soils, 1st crop, mustard, 2nd crop, buckwheat (see Tables X. and XIII.).

It is, however, impossible to explain in this way the results obtained with volatile antiseptics. No chemical reaction can take place between soils and inert bodies like toluene or carbon disulphide vapours, and yet there has been an increase in "availability." The facts are shown very clearly by the analyses in Table VI. (*a*). In this experiment the soils treated with chloroform and carbon disulphide are able to supply the plant with 75 per cent. more phosphoric acid, 50 per cent. more nitrogen,

and 40 per cent. more potash than the untreated soil. Indeed the increased percentage of nitrogen and phosphoric acid in the dry matter of the plants grown on partially sterilised soil suggests that they have more of these two nutrients than is really necessary.

We are compelled to have recourse to a biological hypothesis to explain these facts. Two such hypotheses have already been put forward to account for the increase in productiveness when soil is treated with carbon disulphide, and two others can be added.

A. Koch[1] supposed that carbon disulphide, being a poison, acts in small quantities as a stimulant to the plant causing it to take up more food and so make greater growth. The increase in the first crop might be thus explained, but it is exceedingly difficult to understand how a sufficient amount could remain in these well aerated and frequently watered soils to influence the subsequent crop eight months afterwards. Moreover our experiments with traces—·01 per cent. and ·001 per cent. —of non-volatile poisons, which on Koch's view ought to act as stimulants, have not shown any increase in crop.

Hiltner and Störmer[2] consider the increased productiveness is due to a change in the bacterial flora of the soil. By their method of counting they showed that the first effect of carbon disulphide treatment was to decrease by about 75 per cent. the number of organisms which can live on gelatine, this was followed by an enormous increase, particularly of certain non-liquefying forms. The change in the total number could not be determined owing to the impossibility of counting organisms which do not multiply in gelatine. They suppose that the new flora happens to be more favourable to the production of plant food than the old; as an instance they mention the total destruction of the denitrifying organisms[3].

A third possibility is that partial sterilisation removes a number of organisms which, without benefiting the plant, compete with it for whatever food happens to be present in the soil; when they are killed off a larger amount of food is left available for the plant. This view explains a good deal, but it is difficult to reconcile with the increased absorption of oxygen by the partially sterilised soils. If the number of

[1] *Arbeiten der deutschen Landwirtschafts-Gesellschaft*, Heft 40, 1899.

[2] *Arbeiten aus der Biolog. Abteilung für Land- und Forstwirtschaft am Kaiserlichen Gesundheitsamte*, 1903, Bd. 3, Heft 5. For figures confirming their results see Heinze, *Centralblatt für Bakt. und Parasitenkunde*, 1907, II. abt. 18, 56.

[3] Gerlach, *Bied. Cent.* 1898, 27, 717, had suggested that the increased productiveness was due to the destruction of these organisms.

organisms decreases to the extent indicated by the availability of the plant food there should be a decrease in the amount of oxygen absorbed; the contrary is, however, the case. There are, however, a number of indications that this diminished competition is an important factor.

The fourth hypothesis is an extension of the one formulated by Hiltner and Störmer, and has the advantage of connecting the increased productiveness with the increased oxygen absorption. If we suppose the latter indicates a greater activity of the new bacterial flora it follows that the rate of decomposition in the soil is hastened. The reactions accelerated include the conversion of organised plant and animal matter into humus and mineral substances, the formation of ammonia and the fixation of nitrogen, while the additional carbon dioxide evolved assists the solution of mineral matter. All these reactions contribute to the making of plant food: any increase in the rate at which they take place will increase the productiveness of the soil.

An interesting question that arises is the form in which the nitrogen is taken up. It is generally supposed that a temperature of 90°, or treatment of the antiseptics we used, would be fatal to nitrifying organisms, yet out plants evidently had no difficulty in taking up nitrogen. Beyond watering with sterilised water and keeping the house as free from dust as possible we took no special precautions to prevent reinoculation; it would, however, be difficult to believe that the uniform increases in crop we have had, especially with heated soils, can be due to chance inoculation with nitrifying organisms. We must suppose either that the nitrifying organisms are less easily killed than is generally believed, or that the plants took up some nitrogen compound other than nitrates. Further experiments on this question are required.

Conclusions.

1. Partial sterilisation of soil either by heating to 100° or by treatment with volatile antiseptics which are subsequently removed leads to a marked increase in the amount of oxygen absorbed by the micro-organisms of the soil.

2. The yield of non-leguminous crops is distinctly larger on partially sterilised than on unsterilised soils. Leguminous crops, however, show no increase.

3. Analysis shows that partial sterilisation causes an increase in the amount of nitrogen, phosphoric acid and potash taken up by the crop, and in the percentage of nitrogen and phosphoric acid in the dry matter. In other words it increases the "availability" of these plant foods.

4. The increased availability of the plant food appears to be connected with the modification of the bacterial flora brought about by partial sterilisation. When the soil is heated, however, chemical decomposition also takes place.

NOTES ON THE HOP MILDEW (*SPHAEROTHECA HUMULI* (DC.) BURR.[1]).

By E. S. SALMON, F.L.S.,

Mycologist to the South-Eastern Agricultural College, Wye, Kent.

I. *On the dehiscence of the perithecium.*

It is only quite recently that the perithecium, ascocarp or sporocarp, as it has been variously called, of the *Erysiphaceae* has been shown to possess a regular dehiscence accompanied by the ejection of the ascospores. We find the fact first recorded, I believe, by Worthington G. Smith[2], in 1884. In 1903 I independently observed the phenomenon in the same species, viz. *Erysiphe Graminis* DC., which Smith had studied. Smith's observations seem to have been entirely overlooked until attention was called to them in my paper[3] in 1903, and up to this date the perithecium of the *Erysiphaceae* is described in nearly all text-books as a *cleistothecium* or *cleistocarp*, since it was supposed to remain closed until it decayed and ruptured to permit of the escape of the ascospores.

The following account of the dehiscence of the perithecium of *E. Graminis* is taken from my paper mentioned above. "It was found that the perithecium opened spontaneously; the ascospores were forcibly ejected into the air, and were found germinating in drops of water condensed on the cover of the Petri dish, at a distance of 2 cm. from the perithecium....The dry perithecium is usually concavo-convex, but on absorbing moisture it becomes biconvex. It opens by a horizontal slit, somewhere about the equatorial plane, at one side. The slit gradually extends further and further round, while the upper half of the perithecium, like a lid, becomes lifted up. This circumscissile dehiscence sometimes results in the upper convex half of the perithecium falling away, and the lower exposed basal half remains fixed in the pannose mycelium. The actual dehiscence is in all probability brought

[1] From the Botanical Laboratory, South-Eastern Agricultural College, Wye, Kent.

[2] *Diseases of Field and Garden Crops*, p. 133, 1884.

[3] *Journal of Botany*, p. 161, 1903.

about by the swelling of the mucilaginous cells of the inner wall of the perithecium."

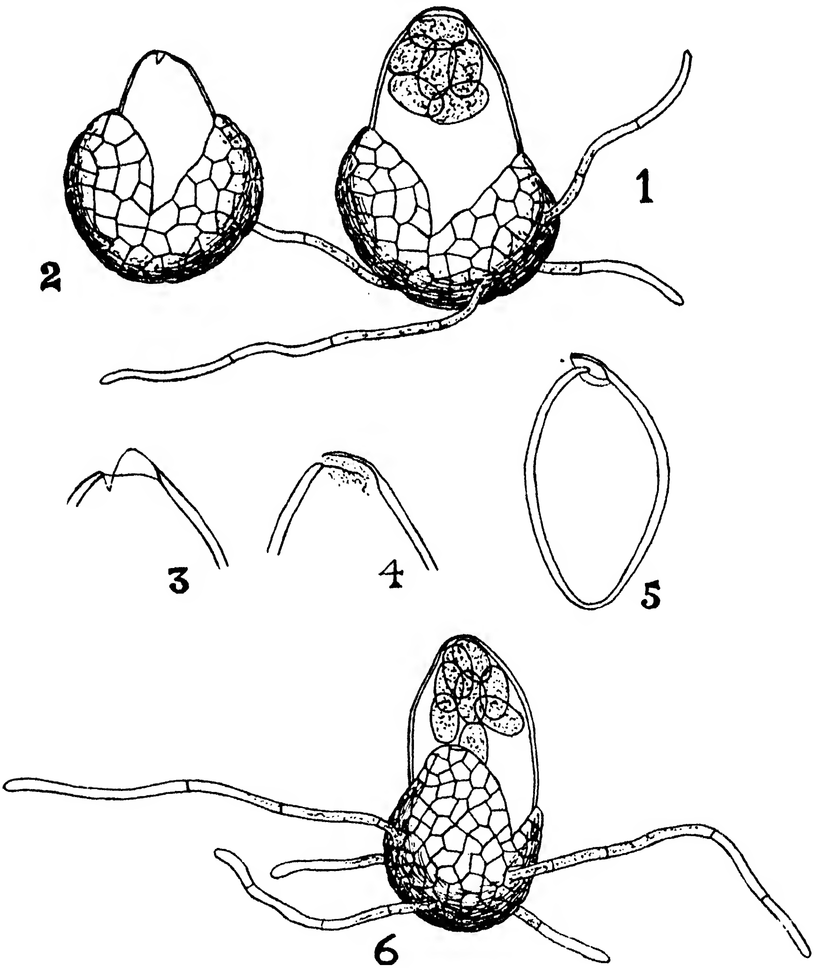
I observed during last spring the dehiscence of the perithecium of the Hop Mildew (*Sphaerotheca Humuli* (DC.) Burr.). The process is somewhat different from what takes place in *E. Graminis*, as described above. The following experiments were made.

Experiment 1. On April 27 some dried moulded "hops" were soaked in distilled water for a few minutes, and then at once (at 11 o'clock) placed on damp blotting-paper in several Petri dishes. By 7 o'clock, that is, after 8 hours, hundreds of ascospores had been ejected from the perithecia, and were visible in the drops of water which had condensed on the lid. In other cases the lid of the Petri dish had been covered on the inside with a film of agar-agar or of gelatine, and to these also many hundreds of ejected ascospores had adhered. In some cases the ascospores had been ejected to a distance of 1·5 cm. The hops used, which were covered with perithecia, had been taken in January from a stack of hopbine and moulded hops standing in a farmyard, and had been kept dry subsequently in the laboratory.

Experiment 2. On May 1 some dried moulded "hops," obtained and treated as above, were soaked for a few minutes in distilled water, and then at once (at 12 o'clock) placed in the open on the surface of some lately dug ground in a garden. The lid of a Petri dish lined on the inside with a film of gelatine was placed just over the "hops." By 3 o'clock, that is, in 3 hours, hundreds of ascospores had been thrown up from the perithecia, and could be seen adhering to the gelatine. The ground on which the moulded "hops" were laid was moist, though not very wet. The weather was showery, sunny at times, and with occasional hailstorms; the wind was cold. A fresh lid was placed over the "hops" at 3 o'clock, and by 6 o'clock numerous further ejections had taken place, and hundreds of ascospores were visible on the gelatine film.

Experiment 3. On May 2 some dried moulded "hops" (taken from a stack in April, and kept dry subsequently) were soaked in water for a few minutes, and then a number of perithecia were lifted off with a needle and placed on wet blotting-paper at the bottom of a Petri dish. A cover slip with a thin film of gelatine was supported on a stand at a distance of 1 cm. from the perithecia, and kept continuously under observation. In 35 minutes the perithecia began bursting, and a number of ascospores were shot up on to the gelatine film. At the end of 1 hour some hundreds of ascospores were visible on the gelatine.

The details of the dehiscence of the perithecium and ejection of the ascospores were then more closely followed. Perithecia were put into a damp chamber or hanging drop of water. In some cases the perithecia which were used were taken straight from a stack of hopbine



E. S. Salmon delt.

and moulded "hops" in a farmyard; in other cases from moulded "hops" and hop leaves which had been kept dry for some months in the laboratory. The results obtained were the same in both cases. After being kept for a short time (in some cases after 1 hour) in a drop of water, the perithecium begins to split by a more or less vertical

slit from the apex (see Figs. 1 and 6). At once the apex of the ascus appears at the slit, and the ascus then rapidly swells by taking up water until it protrudes sufficiently to show the enclosed ascospores (Fig. 6). The ascus continues to swell, until it reaches dimensions often considerably exceeding those of the perithecium in which it was originally contained (see Fig. 1). At this stage the walls of the ascus are in a state of considerable tension; the ascospores are usually collected close under the pore at the apex of the ascus (Fig. 1). In a few minutes the ascus bursts by a small slit at its apex (Figs. 3, 4, 5), and the ascospores are forcibly expelled altogether. The now empty ascus at once contracts and shrinks back into the perithecium, the walls of which come nearer together at the opening. Figs. 1 and 2 show the same perithecium before and after the expulsion of the ascospores; in Fig. 2 the shrunken ascus is seen partly withdrawn into the perithecium. In some few instances, where the perithecium was placed in a hanging drop of water, I have observed the ascus to slip entirely out of the perithecium, soon after the latter has split, before the expulsion of the ascospores. In such cases, which are quite rare, the ascus soon bursts at the apex, as shown at Fig. 5, and discharges the spores,—showing that this process can take place quite independently of the perithecium.

In no instance have I observed any forcible ejection of the ascus from the perithecium, and it seems certain that this does not take place; as a rule the ascus does not even leave the perithecium, but behaves as shown in Figs. 6, 1, and 2. Smith (*l.c.*) has stated that in *E. Graminis* the asci as well as the spores are ejected from the perithecium, but in my observations on that species I was not able to confirm his statement.

It may be mentioned here that I have not succeeded in inducing perithecia of *S. Humuli* collected in the autumn to burst and eject the spores before they have passed through a resting period of some months. The perithecia of *E. Graminis,* as I have shown elsewhere, do not require any resting period.

II. *Inoculation experiments with ascospores.*

Experiment 1. On April 21 some dried moulded "hops" were placed on damp blotting-paper in a Petri dish. By April 23 the perithecia had burst, and thrown up many hundreds of ascospores, which could be seen floating in the little drops of water condensed on the lid of the dish. Many of these spores had already commenced

to germinate. A number of these ascospores were now transferred to the upper surface of a marked leaf of a young (seedling) hop. Seven days later a small patch of *Oidium*, already powdery with conidia, was visible on the marked leaf, at the spot where the ascospores had been sown.

Experiment 2. On April 29 some hundreds of perithecia which had just arrived at the stage of bursting and ejecting spores, were sown on the upper surface of a marked leaf of a seedling hop, and also of a young plant of *Potentilla reptans.* By May 6 a fairly large, powdery patch of *Oidium* had appeared on the hop leaf at the place where the perithecia had been sown. By May 12 this had developed into a large, very conspicuous, white, densely powdery patch. No trace of infection resulted on the *P. reptans.*

Experiment 3. On May 1 a young leaf of a seedling hop and a young leaf of a plant of *P. reptans*—in both cases attached to the plant—were laid upon some moulded "hops" which at the time were ejecting ascospores from the perithecia. The leaves remained in this position for 24 hours, when the two plants were placed under a bell-jar. At the end of 9 days, a vigorous, powdery patch of *Oidium* appeared on the leaf of the hop. No trace of infection resulted on the leaf of *P. reptans.*

Experiment 4. Another experiment, exactly similar, started on May 2, gave the same results, viz. the infection of the hop, but not of *P. reptans.*

Experiment 5. On May 11 drops of water containing many hundreds of recently ejected ascospores were placed on a leaf of a young hop, and on a leaf of *Spiraea Ulmaria,* in each case within a marked area on the leaf. On May 21 some delicate radiating mycelial hyphae and a few scattered conidiophores were visible at the marked place on the hop leaf. On May 26 a conspicuous powdery patch of *Oidium* had developed on the hop leaf at the marked place only. No trace of infection resulted on the *S. Ulmaria.*

Experiment 6. On May 12 conidia were taken from the *Oidium* patch produced on the hop leaf in the above experiment, and sown on a marked leaf of a hop and on a marked leaf of *S. Ulmaria.* By May 26 a powdery patch of *Oidium* had been produced at the marked place only on the hop leaf; no trace of infection occurred on the *S. Ulmaria.*

We may conclude from the above inoculation experiments that the mildew on the Hop is, at least to a certain extent, a distinct "biologic

form," since it is unable to infect *P. reptans* and *S. Ulmaria*, both host-plants of the morphological species *S. Humuli.* I have previously shown[1] that the conidia of the form of *S. Humuli* on *P. reptans* are unable to infect *Alchemilla vulgaris*, *A. arvensis*, *Fragaria* sp., *Spiraea Ulmaria*, *Agrimonia Eupatoria*, and *Poterium officinale*,—all of which are host-plants of the species. The same specialisation of parasitism has been proved (*l.c.*) to exist in the case of the variety *fuliginea* of *S. Humuli.*

All the evidence, then, shows that the morphological species *S. Humuli* consists of a number of specialised "biologic forms," each of which is incapable of infecting any of the host-plants of the others. The point is of some economic importance. It has been the habit of agricultural writers who have treated of "hop mildew" or "mould" in connection with the cultivation of hops, to call attention to the fact that this mildew lives on a number of wild plants, such as "Meadow Sweet" (*Spiraea*) and Avens (*Geum*); and then, having assumed that the mildew could pass from these plants on to cultivated hops, these writers have advised the hop-grower to spray, or to get rid of, these "mouldy" wild plants[2].

From our present knowledge of the specialisation of parasitism of *S. Humuli*,—as well as of the high degree of specialisation which has been proved to exist in the *Erysiphaceae* generally[3],—the hop-grower should not be recommended to take any special steps to deal with mildewed weeds in or near the hop-garden. The whole of the evidence to hand strongly supports the view that hop "mould" is due to one special "biologic form" confined to *Humulus Lupulus* and *H. japonicus*, and that consequently hop-growers have nothing to fear from other forms of *S. Humuli* growing on wild plants. It must be pointed out, however, that until inoculation experiments with all the forms of the fungus on the thirty or more host-plants[4] on which it occurs in this country have been carried out, the bionomics of the morphological species *S. Humuli* must remain only partly known.

[1] *The New Phytologist*, III. p. 111, 1904.

[2] See *e.g.* Percival, *Agric. Botany*, p. 732, 1902; *Journ. Bath and West of England Soc.* XV. p. 78; Myrick, *The Hop*, p. 152 (1899).

[3] See Salmon, E. S., "Recent Researches on the Specialisation of Parasitism in the *Erysiphaceae*," *The New Phytologist*, III. p. 55, 1904; *l.c.* p. 109, where a bibliography of the subject is given.

[4] See Salmon, E. S., Monograph of the Erysiphaceae, 1900, and "Supplementary Notes," *Bull. Torr. Bot. Club*, 1902.

STUDIES OF SOIL MOISTURE IN THE "GREAT PLAINS" REGION.

BY F. J. ALWAY, PH.D.

Professor of Agricultural Chemistry, University of Nebraska.

THE "Great Plains" region, the western portion of what is commonly known as the prairies of North America, extends from the northern parts of Texas and New Mexico into the south of Alberta and Saskatchewan. It forms a belt stretching from the foothills of the Rocky Mountains eastward for some two or three hundred miles, the eastern boundary being ill-defined and fluctuating with the cycles of wet and dry seasons. The region is characterised by a dry atmosphere with strong winds, low minimum temperatures in the winter and very high maxima in the summer, with a comparatively small rainfall, most of which occurs during the summer months. A similar total precipitation of 15 or 16 inches per annum occurs west of the Rocky Mountains, but falls chiefly during the winter months and is then found to be sufficient for the profitable production of grain crops. In the "Great Plains" region in Saskatchewan the usual custom is to take a bare fallow one season in three in order to accumulate the rainfall for the benefit of the two following grain crops.

This rough rule, which does not take into account the variable character of the seasons, can advantageously be replaced by determinations of the water content of the soil before the growing season commences, so as to measure the amount of water available for the crop.

Before however any judgement can be formed on this point it is necessary (1) to take samples down to the depth of six feet, (2) to determine not merely the total water but the amount of "free" water in the soil.

As to the depth to which samples should be taken it is found that in Saskatchewan on the clay soil generally the roots of cereals and grasses penetrate to the depth of four to six feet; below this depth

the limited rainfall is rarely likely to penetrate nor will the moisture from the lower layer be elevated by capillarity to any effective extent. By the "free" water in the soil is understood the measured water less the "hygroscopic coefficient" of the soil as defined by Hilgard[1], *i.e.* the percentage of moisture which a thin layer of soil can absorb from an atmosphere kept saturated with water vapour. It is certain that plants cannot utilise any of the hygroscopic water in a soil for growth, though data are lacking as to how near this point the soil water can be reduced without killing the plant; at any rate the "free" water measures the maximum amount that can be available for the plant. As the "hygroscopic coefficient" of the soils of this region examined by the author varies between 4 and 14 it is obvious that determinations only of the water present in the soil would give very erroneous measures of the relative amounts of water available for the crop.

The author's observations were made in July 1904 and Sept. 1905 on the Canadian Experimental Farm at Indian Head, and at Moose Jaw, Saskatchewan. The samples were taken with an auger, 1½ inches in diameter; they were weighed in the field and then despatched to the laboratory in Nebraska for determination of the dry matter and the "hygroscopic coefficient."

1. *Uniform soil—effect of crop v. fallow.*

Soil samples were taken on Sept. 9th, 1905 from two adjoining fields at Moose Jaw, one being under an unusually clean summer fallow, the other having just yielded 40 bushels of wheat and 3200 lbs. of straw per acre. In 1903 both fields had been under small grain crops, and in 1904 the first field had been fallowed while the second had carried oats or wheat. In 1904 the rainfall had only been 7·90 inches, but in 1905 as much as 15·32 inches had already fallen, of which only 0·78 inch had fallen between July 30th of that year and the date of sampling.

Table I. shows both total and free water in each successive foot of soil down to the depth of six feet: the soil is a lacustral clay of a heavy type, the mechanical analysis of which is given in Table II. Determinations of the "hygroscopic coefficient" and of the carbon dioxide evolved on treatment with acid of these and other samples show its uniformity over a considerable area.

[1] University of California Agric. Exp. Sta., *Circular* 6 (1903), p. 17.

TABLE I.

Water in fields on uniform lacustral clay.

	Foot section	Percentage of total water	Hygroscopic coefficient	Percentage of free water	Field notes on condition of soil
Field G, Fallow	First	23·6	13·9	9·7	Moist
	Second ...	36·8	12·2	24·6	Very moist
	Third......	33·2	11·7	21·5	,, ,,
	Fourth ...	27·7	11·8	15·9	Moist
	Fifth	26·0	11·9	14·1	,,
	Sixth......	25·2	11·5	13·7	,,
	Average...	28·7	12·1	16·6	
Field F, Wheat	First	22·2	13·6	8·6	Moist
	Second ...	18·6	12·7	5·9	Dry
	Third......	18·0	11·8	6·2	,,
	Fourth ...	23·5	11·9	11·6	Moist
	Fifth	24·4	11·7	12·7	,,
	Sixth......	25·0	11·4	13·6	,,
	Average ...	21·9	12·2	9·8	

The fallow field G contained within the first six feet of soil an average of 6·8 per cent. more water than the adjoining field F, which had just brought a crop of wheat to maturity. The wheat roots had not drawn as heavily upon the moisture of the fourth and fifth feet as upon that of the second and third feet, nor had the season's rainfall been sufficiently conserved to raise the moisture content of the soil below the third foot to near its limit. It should be noted, however, that the season of 1905 with an exceptionally well distributed rainfall had not been favourable for the reduction of the soil moisture to its minimum, and also that the rainfall of the previous year had not been sufficiently heavy at Moose Jaw to raise the moisture content of the first four feet to its maximum, even if surface evaporation had been entirely prevented. In the case of the fallow field about half of the rainfall of the season had been stored, chiefly below the first foot.

When the amounts of total water in the fields, F and G, are compared, the relative moisture conditions are shown correctly, thus rendering it not necessary to make determinations of the hygroscopic coefficient on all the different samples when only adjacent fields are under comparison. The necessity of deep sampling, however, is very apparent. If samples had been taken to a depth of only one foot,

very little difference in the moisture content of the two fields would have been found, although there was, in reality, enough difference to account for a difference in yield in the following year, as shown by another experiment on the same farm, of twenty bushels of wheat per acre.

TABLE II.

Mechanical analysis of specimens of the two soil types.

	Boulder clay per cent.	Lacustral clay per cent.
Gravel, 2·0 to 1·00 mm.	1·7	0·0
Coarse sand, 1·0 to 0·5 mm.	5·2	0·01
Medium sand, 0·5 to 0·25 mm.	5·6	0·01
Fine sand, 0·25 to 0·10 mm.	14·1	2·2
Very fine sand, 0·10 to 0·05 mm.	12·3	6·7
Coarse silt, 0·05 to 0·016 mm.	16·2	19·0
Fine silt, 0·016 to 0·005 mm.	19·5	22·6
Clay, below 0·005	26·1	48·2
Total	100·7	98·8

2. *Mixed soil.*

In the fields of the Indian Head Experimental Farm from which the following samples were taken two types of soil are to be found; a lacustral clay in every way similar to that described at Moose Jaw forms the surface layer and possesses a thickness varying from a few inches to three feet, below which it is underlaid by a boulder clay. The latter soil, as shown by the mechanical analysis in Table II., is of much lighter character, it contains less clay and more carbonate of lime, and possesses a much lower hygroscopic coefficient, 4—7 °/₀ instead of 11—15 °/₀ for the lacustral clay.

In Table III. the water content of the soils from five different fields is brought together for comparison, samples having been taken both in 1904 and 1905; only the total water and the free water figures are shown, the latter being printed in italics, it being understood that in each case the hygroscopic coefficient has been determined and deducted from the total water in order to arrive at the free water.

During 1904 the summer rainfall had been light, amounting only to 6·67 inches between April 1st and the time of sampling, and of this 1·15 inch had fallen on July 12th, 13th shortly before sampling. During 1905 the rainfall up to the end of August had been 13·77 inches, of which 1·28 inch fell on Aug. 30th, 31st, immediately before sampling.

TABLE III.

Water in soils of mixed origin at Indian Head.

Foot Section	D Fallow Total	D Fallow Free	B Fallow Total	B Fallow Free	A Grass Total	A Grass Free	C Oats Total	C Oats Free	E Fallow Total	E Fallow Free
July 25th, 1904										
First	34·4	*20·9*	29·4	*17·4*	19·0	*7·5*	20·0	*7·3*		
Second ...	29·7	*16·2*	14·9	*11·0*	14·8	*4·5*	22·4	*9·2*		
Third ...	29·5	*16·6*	16·4	*11·7*	13·9	*5·8*	21·6	*8·1*		
Fourth ...	20·3	*12·9*	17·4	*11·9*	13·6	*6·7*	16·1	*11·5*		
Fifth	15·2	*8·6*	21·8	*14·2*	12·0	*5·0*	15·1	*10·9*		
Sixth	19·2[1]	*12·6*	19·6	*11·6*	15·4	*6·5*	15·9	*10·0*		
Mean ...	24·7	*14·6*	19·9	*12·9*	14·8	*6·0*	18·5	*9·5*		
September 5th, 1905	Wheat		Oats		Grass		Wheat		Wheat	
First	28·9	*16·1*	22·4	*10·6*	25·9	*15·9*	28·8	*17·9*	26·4	*16·9*
Second ...	22·7	*9·6*	16·7	*5·9*	20·3	*9·4*	19·8	*10·1*	14·8	*7·9*
Third......	20·9	*8·6*	9·2	*4·5*	20·9	*10·0*	10·9	*6·1*	15·4	*9·6*
Fourth ...	11·8	*6·3*	9·6	*5·2*	15·4	*10·7*	17·1	*11·7*	13·2	*7·4*
Fifth	16·4	*10·9*	14·4	*10·0*	15·1	*10·2*	18·4	*12·9*	15·6	*10·0*
Sixth	19·2	*12·6*	17·8	*12·6*	13·6	*9·1*	19·0	*14·6*	21·8	*11·8*
Mean ...	19·9	*10·6*	15·0	*8·1*	18·5	*10·9*	19·0	*12·2*	17·9	*10·6*

The determinations of hygroscopic coefficient indicate that the lacustral clay extended to a depth of three feet in field D, a foot only in field B, about two feet in A, and about three feet in C, while the samples from field E were taken from a ridge of the boulder clay.

In field D the determinations in 1904 show plenty of free water under the fallow; in 1905 despite the growth of a crop of 32 bushels of grain and 3700 lbs. of straw per acre, the subsoil had been by no means completely exhausted of its free water. The moistness of the first two feet was due to the rains subsequent to the cutting of the crop.

Field B gave very similar results to D; uniformly moist under the fallow in 1904, it has been considerably but not completely dried by the oat crop of 1905 (95·3 bushels of grain and 5960 lbs. of straw per

[1] This sample was lost, so the figure for the sixth foot in 1905 has been adopted.

acre) in the second, third and fourth feet, but the fifth and sixth feet were still moist and the surface had regained moisture by the recent rains.

Field A was covered with Western Rye Grass (*Agropyrum tenerum*) and was cut for hay on July 18th, 1904.

During this dry season the grass had very thoroughly exhausted the soil of water; down to the sixth foot there was always less than 7 per cent. of free water, the slight excess in the top foot being due to the rainfall of July 12th, 13th. Roots were found to a depth of 5′ 6″, and in the sixth foot while the upper layers of soil were quite dry and powdery the lowest layer was moist and sticky. In 1905 it is clear that but little of the rainfall after mowing on July 13 had been transpired by the grass, so that the subsoil down to six feet had become moist.

Field C, which adjoins D, is continuously cropped with cereals, and at the time of sampling in 1904 was showing the effects of the drought more than any other field on the farm, the straw being short and yellow. Notwithstanding this the soil appeared moist at all depths and showed more free water than several of the other fields.

When these results are considered together it is seen that the total amounts of water in the soil would lead to very erroneous judgements as to its capacity for supporting a crop. For example field F in 1905, which had just brought a heavy crop of wheat to maturity after a month of almost rainless weather, held more water in the first six feet of soil than did B in 1904, after a season's fallowing. Field C also in 1904, though showing serious effects of drought, contained only 1·4 per cent. less moisture than did B on the same day. Yet in the following year the yield of grain and straw per acre was 9200 lbs. on B and 2160 lbs. only on C.

When the fields are arranged in the order of their amount of free water they are given more nearly their proper place, all the fallows being placed before the grain and grass fields.

A comparison of the fields on the basis of the amount of free water in the first six feet assumes that a definite value is to be attached to a certain percentage of free water, independent of the kind of soil and of the portion of the six feet in which it occurs. It is evident from Hilgard's work, however, that a higher percentage of free moisture is required on clay than on sandy soils in order that plant growth may continue. The data in the above tables indicate that wheat and oats may reduce the free water to about 6 per cent. through

the first three feet of lacustral clay and to 4 or 5 per cent. through the first four feet of boulder clay. The fourth and fifth feet, respectively, while not being exhausted to the same extent as the soil above, give up part of their free water. The fifth and sixth feet, respectively, seem to furnish a small and, perhaps, negligible portion of their water to oats and wheat. In the case of the boulder clay, a native grass is able to exhaust the fifth and the upper half of the sixth foot as completely as the two cereal crops do the fourth.

In Table IV. an attempt has been made to show, as well as the limited amount of data will permit, the quantities of water, in the different fields, available for wheat and oat crops. It has been assumed that the lower limit of free moisture in the boulder clay is 4·5 and in the lacustral clay 6 per cent. The free water less this 4·5 or 6 per cent. is for convenience at present designated the "x water." It has also been assumed that all the water of the first three feet and half of that of the fourth foot are available in the fields at Moose Jaw and that the x water of four and a half feet is available at Indian Head.

If the fields are arranged in the order of the amount of x water within reach of wheat and oat roots, and if they are also ranked according to the writer's field notes concerning the moistness or otherwise of each soil section, it is interesting to find that both methods show the fallows grouped at the top and the three driest fields at the bottom. Thus the judgement of an agriculturist formed without the use of any apparatus except an auger is more reliable than a determination of total moisture when the hygroscopic coefficient is not also determined.

The ordinary prairie farmer, provided with a six foot auger, can form a fair estimate of the moisture condition of his fields before the spring is sufficiently advanced to permit of seeding, and will thus be in a position to decide intelligently whether to sow grain upon his stubble fields or to summer-fallow them. At present his decision is governed by the rule "one year of fallow followed by two years of grain." Normally the soil is dry enough in the spring to justify the rule, but in such years as 1899 and 1901, when the preceding autumn had been exceptionally wet (nine inches of rain falling after the ripening of the cereal crops), the stubble fields have probably been nearly as wet to a depth of five feet as the fallows were.

TABLE IV.

The amount of water in different fields that is probably available for the support of normal growth of wheat and oats, expressed as percentage of dry soil.

Foot section	D July 1904	G Sept. 1905	B July 1904	C Sept. 1905	A Sept. 1905	E Sept. 1905	D Sept. 1905	C July 1904	B Sept. 1905	F Sept. 1905	A July 1904
First	14·9	3·7	11·4	11·9	9·9	10·9	10·1	1·3	4·6	2·6	1·6
Second	10·2	18·6	6·5	4·1	3·4	3·4	3·6	3·2	1·4	0·0	0·0
Third	10·6	15·5	7·2	1·6	4·0	5·1	2·6	2·1	0·0	0·2	1·3
Fourth	8·4	4·9	7·4	7·2	6·2	2·9	1·8	7·0	0·7	2·8	2·2
Fifth	2·1	0·0	4·8	4·2	2·8	2·7	3·2	3·2	2·2	0·0	0·2
Average of 5 feet	9·2	8·5	7·5	5·8	5·3	5·0	4·3	3·4	1·8	1·1	1·1
Tons per acre	690	637	562	435	397	375	325	255	135	83	83
Inches of Rain	6·1	5·6	5·0	3·9	3·5	3·3	2·9	2·2	1·2	0·6	0·6

3. *The depth to which the frost penetrates.*

Both English and American authorities have advanced or accepted, in explanation of the large yields of grain in southern Saskatchewan, the theory that the frost penetrates to a great depth during the winter, and during the summer months furnishes a constant supply of moisture to the growing grain.

Sir William Crookes[1] has stated:

"The fertility of the North-west Provinces of the Dominion of Canada is due to an exceptional and curious circumstance. In winter the ground freezes to a considerable depth. Wheat is sown in the spring, generally April, when the frozen ground has been thawed to a depth of three inches. Under the hot sun of the short summer the grain sprouts with surprising rapidity, partly because the grains are supplied with water from the thawing depths. The summer is too short to thaw the ground thoroughly, and gate posts or other dead wood extracted in the autumn are found still frozen at their lower ends." Storer[2] in his textbook on Agriculture quotes, as an illustration

[1] Presidential Address, British Association (1898), Report, p. 8; *The Wheat Problem*, p. 22 (1900).

[2] *Agriculture* (7th Ed.), vol. I. p. 144.

of how melting ice may serve as a useful store of moisture in certain situations and for certain crops, that in Assiniboia[1], "even in late July some of the soil still holds the winter's frost at a depth of several feet below the surface. This underground layer of frozen earth is believed to explain the wonderful fertility of the soil, as the frost, in gradually coming to the surface during the summer months, creates a moisture which, meeting the warmth from above, forms a kind of natural hot bed. This moisture counteracts the scarcity of rain during the spring and summer and accounts for the grain being forced with such amazing rapidity after the late sowing."

The winter of 1903—1904 was the coldest that had occurred in twelve years in Saskatchewan, the spring was late, and the early part of the summer cool. During the second week in July 1904, the writer made borings to a depth of nine feet in the most exposed situations to be found in the vicinity of Moose Jaw. There was no trace of frost, although the soil, at a depth of six feet, was distinctly chilly to the touch; both above and below it was decidedly warmer. In the same district on the 1st of April in 1906 numerous borings were made through the frozen soil. In the exposed gardens and fallows the frost had penetrated from 4½ feet to 5½ feet. On the stubble it had reached a depth of only 3½ feet.

Not even the chilly layer of subsoil was found in the borings made at Chaplin and at Indian Head in 1904 or at the latter place and Moose Jaw in September 1905.

It is probable that the frost is all out of the ground before the 1st of July of every summer, unless it be on the edge of the forest country, where the drifting leaves may serve to prevent the thawing of the soil.

It is possible that the low winter temperature may assist in the transfer of water, by distillation, from the lower moist zone to the upper, if the latter has been dried out by a crop. The frost, however, seems to have nothing to do with the exceptionally large yields of grain which are obtained only on summer-fallowed land or its equivalent, and which bear no relation to the temperature of the preceding winter.

SUMMARY AND CONCLUSIONS.

1. All determinations of soil moisture should be made to a depth of from four to five feet for wheat and oats, and to a depth of six or seven feet for grasses.

[1] Now the southern portion of the Province of Saskatchewan.

2. Unless all the soil under consideration is very uniform, determinations of the hygroscopic coefficient are indispensable. The determination of this value is extremely important even where the soil is uniform.

3. The storage capacity for available water of the two soil types studied, may be placed at from five to seven inches of rainfall for wheat and oat crops.

4. A better idea of the moisture conditions of the soil at Indian Head may be obtained from a casual examination in the field than from the drying and weighing of the samples, unless the hygroscopic coefficient is considered.

5. The moisture stored in the subsoil during the previous summer, and not the frost of the preceding winter, is the cause of the high yields of wheat and oats obtained in southern Saskatchewan.

6. The soil of southern Saskatchewan does not remain permanently frozen at any depth.

7. Investigations of the moisture conditions to a depth of only 12 to 16 inches are of no value and may often be entirely misleading.

THE NITROGEN COMPOUNDS OF THE FUNDAMENTAL ROCKS.

By A. D. HALL and N. H. J. MILLER.

Rothamsted Experimental Station.

It has been shown[1] that many rocks, when samples are taken from such a depth as precludes all possibility of weathering, contain nitrogen in amounts comparable to those present in soil, especially when the strata consist of indurated clays like the Lower Lias, Oxford, Kimmeridge or London Clay. Since the rocks also contain carbon the nitrogen is without doubt of organic origin; the carbon and nitrogen compounds represent the humus present in the clay when it was deposited, and are in fact the mineralised remains of that organic matter. When soil is produced by the weathering of such rocks these carbon and nitrogen compounds will remain wholly or in part in the soil and may there account for a considerable proportion of the total nitrogen they contain. It thus becomes of some importance to ascertain if this nitrogen is ever likely to become available for the plant by the normal processes of bacterial oxidation, or whether it has passed into such a state of combination as to be susceptible of no further change under such conditions as prevail in the soil. If the compounds are too mineralised or bituminised to be attacked by bacteria it would explain the fact that much of the nitrogen present in soils seems to remain permanently beyond the reach of plants. For example the soil of Broadbalk wheat field at Rothamsted, which had been cropped continuously with wheat for 50 years without the addition of any manure, still showed in 1893 very nearly 0·1 per cent. of nitrogen, equivalent to about 2500 lbs. per acre in the top 9 inches of soil; yet the average wheat crop on this plot only contains about 17 lbs. of nitrogen, of which 5 lbs. is supplied annually by the rain, without taking into account any further additions by the action of *Azotobacter* and kindred organisms.

It was therefore decided to subject certain of the deep-seated rocks, the carbon and nitrogen of which had already been determined (*loc. cit.*), to the action of soil bacteria, to ascertain if any measurable amount of nitrates would be produced. A kilogram or so of the rocks was reduced to powder and mixed with sufficient ignited coarse sand to keep them open, and a little potassium phosphate and calcium and magnesium

[1] Miller, *Quart. Journ. Geol. Soc.* 1903, **59**, 133.

sulphates to provide mineral food for the bacteria; when necessary 1 per cent. of calcium carbonate was also added and the dishes containing the mixture were moistened and placed under bell-jars over water and stored. At starting the mixtures were inoculated with the filtered extract from a fertile garden soil, occasionally the mass was stirred and a fresh inoculation given, the nitrogen in the added soil extracts being determined each time. After fourteen months' exposure the nitrates were washed out of the mixtures and determined (Series I). In all cases nitrates were found, in amounts beyond any possible experimental error wherever the rock itself contained an appreciable amount of nitrogen. The trial was repeated on fresh rocks which were again exposed for a year with similar results (Series II). At this stage it was found that some of the rocks contained appreciable amounts both of nitrates and of ammonium salts, which would yield ammonia on distillation with magnesia and which when oxidised would account for more than the nitrates found. Accordingly a fresh series of rock powders was made and distilled in small portions at a time with magnesia and water under reduced pressure in order to get rid of compounds of ammonia. The mass was dried to remove the excess of water, and then inoculated and exposed as before, this time for a period of three months only (Series III). Since the nitrates were not removed with the ammonia before the oxidation began, their amount was determined in fresh samples of the rocks. Again more nitrates were found than were present in the original rock, so that there can be no doubt but that the nitrogen compounds of the fundamental rocks, other than the ammonium compounds, can be slowly attacked by bacteria and converted into nitrates.

The Table gives particulars of the rocks examined—percentages of carbon, total nitrogen, and nitrogen as ammonia and nitrates, also the amount of nitrates formed in each of the three experiments.

The authors conclude that the nitrogen compounds of the soil are not wholly of recent origin, but have in part been derived from the rock out of which the soil has been formed by weathering. In some of the clay soils the proportion of nitrogen due to the original rock is likely to be considerable, which may account for the comparative infertility of many clay soils which by analysis appear to be rich in nitrogen. Such nitrogen compounds are however to some slight extent slowly attacked by bacteria and yield nitrates available to the plant.

The authors are much indebted to Mr H. B. Woodward, F.R.S., of H.M. Geological Survey, for the samples of deep-seated rocks numbered 5, 9, 10, 11, 12, and 13.

Carbon and Nitrogen in unweathered rocks and nitrates produced therefrom.

Number of Soil	Formation	Depth at which Sample was taken in feet	Organic Carbon %	Nitrogen %	C/N ratio	Nitrogen mgs. per kilo.		Nitrates formed mgs. Nitrogen per kilo. after deducting nitrogen in soil extract and nitrates in the rock before exposure		
						as Ammonia	as Nitrate	Series I	Series II	Series III
1	Lower Bagshot Sand, Weybridge ...	18—20	0·02	·00384	5·4	—	—	0·52	—	—
2	Upper Greensand, Farnham, Surrey	30	0·032	·00718	4·5	—	—	0·55	—	—
3	Folkestone Beds, Brabourne, Kent...	20	0·019	·00453	4·2	—	—	–0·2	—	—
4	Lower Greensand, Sevenoaks	30	0·076	·00881	8·7	—	—	2·2	—	—
5	London Clay, London	130	0·391	·041	9·5	9·5	0·49	4·56	—	—
6	Gault Clay, Nackholt, Kent............	18	0·427	·0415	10·3	14·0	0·69	10·51	—	—
7	Weald Clay, Pluckley, Kent	30	0·135	·0647	2·1	19·6	0·42	9·84	—	1·28
8	Carboniferous Shale, Barnsley.........	1236	1·938	·137	14·1	25·2	0·35	9·70	—	1·03
9	Lower Gault, Dover	280—400	0·172	·0325	5·3	—	2·01	—	0·41	—
10	Oxford Clay, Dover	920	0·548	·0528	10·4	—	0·72	—	5·45	1·60
11	Kimmeridge Clay, Welton, Lincs. ...	246	2·139	·107	20·0	—	1·25	—	0·79	0·60
12	Kimmeridge Clay, Dover	570	0·387	·0455	8·5	—	0·39	—	2·78	1·92
13	Lower Lias, Mickelton, Glos.	700	1·120	·0803	13·9	—	1·08	—	1·21	1·84
14	Clay with Flints, Harpenden	10	0·577	·0294	19·6	—	0·61	0·62	—	—
15	,, ,, ,, ...	Surface soil	—	—	—	—	—	8·40	—	—
16	Coal	—	—	1·740	—	—	—	—	–0·5	—

NOTES.

1. A grey coarse sand.
2. Pale grey fine sandy rock.
3. Coarse yellowish sand.
4. Fine yellowish sand.
5. Solid grey clay from the Tube Railway workings below Covent Garden.
6. Solid dark green clay.
7. Close grey and red mottled clay forming a red powder.
8. Hard grey shale from the Wath-on-Dearne pit.

9, 10, 12. Hard grey clays from the coal pit shafts at Dover.

11. Hard grey clay.
13. Hard grey clay
14. Reddish sandy brick earth.
15. Surface soil from the unmanured plot on the Barnfield, first extracted with water to remove nitrates and then sterilised by heating to 110° on two successive days.

MENDELIAN STUDIES OF EGYPTIAN COTTON.

By W. LAWRENCE BALLS, M.A.,

Botanist to the Khedivial Agricultural Society, Cairo.

Introduction.

THE Khedivial Agricultural Society of Egypt recently voted a capital expenditure of £2000 towards the establishment of a Mendelian Experiment Station for the study of heredity in cotton.

This Station will form the centre of a system of Seed Stations in various parts of Egypt; the strains of cotton sent out from the Central Station will be tested in these for their agricultural utility, and propagated for distribution to the cultivators.

The project is based on the researches of the last three years; I propose to abstract the results of these researches in the present paper. Various publications in the Year-Books of the Society for 1905 and 1906 give detailed accounts of the preliminary work up to the end of the latter year. The present account will deal only with Genetics, summarising the subject up to date.

Previous Work.

There is not much information of value among the old literature. Some of the work which has been accomplished on the strength and quality of cotton fibre has been of use to me, as have also the accounts of variety-trials. Owing to the absence of any individualistic conception in the published accounts of cross-breeding no evidence of any kind could be obtained from them, even as to the direction of dominance.

The only paper on Mendel's Law in cotton, other than the present writer's preliminary accounts[1], is by Fletcher[2]. Experiments are being conducted by Fyson in Madras, but nothing has been published[3].

[1] This *Journal*, Vol. II. Pt 2; *Khedivial Agricultural Society's Year Book* for 1906.

[2] This *Journal*, Vol. II. Pt 3.

[3] Since going to press an important contribution by H. Martin Leake has appeared in *Jour. and Proc. Asia. Soc. Bengal*, IV. 1. 1908. *Vide inf.* pp. 357, 365, 368.

Initial Problems.

It is widely believed in Egypt that the cotton crop is deteriorating in yield and quality. In the absence of full historical evidence and samples no definite proof of this can be given, but the yield per acre is certainly decreasing. The decreased yield is due to various causes, some of which are outside our province, such as meteorological changes, elevation of the water table, arrest of nitrification through over-watering, insect pests, etc.

In addition to the depreciation of the general crop the cotton experts recognise that each variety deteriorates after it has been grown for a few years. For instance, the original Afifi cotton was composed of strains which matured early in the season, and finished their growth by the time that the modern Afifi is yielding its second picking. During the last two years it has become evident to the grading experts that Yannovitch, the most recent of those varieties which are extensively grown, is beginning to degrade.

Very little was formerly known about the crop from the analytical standpoint; it was even thought possible that the "Hindi" weed cottons might be transmuted into Egyptian plants, and *vice versa*, so that the first necessity before me was to obtain approximate knowledge of the three following subjects:

A. The extent of the fluctuation in the various characteristics of the individual plant, especially of the seed and lint.

B. The number and diversity of the various forms which presumably constituted the commercial varieties.

C. The gametic composition of the individual plants; otherwise expressed, the amount of cross-fertilisation under field conditions. The answer to this problem would be of the utmost importance; its effects would be felt throughout the whole research, in the applied work even more than in the purely scientific portion.

The next stage of the research was to determine the allelomorphs involved in cotton hybrids. Some of these were determined from natural hybrids when investigating the gametic composition of individuals, but the systematic attempt to elucidate Mendel's Law is being made on crosses between cottons differing in many characters, such as Egyptian and American Upland. This method of attack, contrary to the Mendelian principle of investigating simple cases first, was necessitated by the urgency of practical requirements. Nothing has been lost by it, since we are to have facilities for dealing with the large populations which the method requires.

Practical problems arising from the application of the knowledge acquired included the selection of plants from the existing crop, and the purification of their splitting-forms; the synthesis of new cottons with earlier maturity, heavier yield, longer, more uniform and better staple, etc.; and the planning of methods for propagating pure strains without contamination[1].

PART I. ANALYSIS OF THE CROP.

The initial determination of the nature of the crop was made from study of the seed, with its attached lint.

The usual method of examination of cotton seed is to comb the lint out into two wings on either side[2]. This method does not demonstrate a very important character, *i.e.* the relative length of the lint on various parts of the seed. The plan adopted throughout these experiments has been to comb the lint out into a disc, parting the hairs along the raphe.

Measurements were made from the surface of the seed coat to the edge of the disc of hair. This edge is not quite definite, so that a subjective error of a millimetre is involved.

Strength was tested by breaking fibres between the fingers in the usual manner, but it would be well to point out here that strength of fibre is mainly dependent on the suitability of the plant to its environment, though the average breaking strains of various kinds of cotton are by no means the same.

SECTION 1.

Fluctuation of Seed Characters.

A great deal of description of various kinds of seed cotton has been written, but, as we shall see later, the fact of two cottons bearing the same variety-name is no guarantee of their bearing similar characters. There was no available evidence as to the constancy or otherwise of the various characters, when pure strains were employed.

The following conclusions were drawn in the first instance from comparison of seed formed on the same plants at various stages of growth. The first flowers appear near Cairo in June, and the first bolls are mature in the end of August, while some bolls succeed in opening as late as December. Examination of our meteorological records will show

[1] The writer, *Proc. Cairo Sci. Soc. Cairo Sci. Jour.* No. 17, 1908. With discussion on causes of crop deterioration in Egypt.

[2] *E.g.* H. J. Webber, *Yr. Bk. U. S. Dep. Agr.* 1902, Plate XLIV.

that the first and last bolls develop under very widely different conditions of environment.

Subsequently, the conclusions drawn in this way were confirmed by the study of isolated strains[1], pure in respect of one or more seed characters, which were grown for four consecutive years under varying conditions of cultivation.

Descriptive characters of the ginned seed.

Fuzz, colour. The colour of the short hairs which underlie the cotton lint may be green, brown, or white.

Green fades to brown in sunlight, but some strains retain their colour more firmly than others. White fuzz is not known to me in Egyptian cottons, but in other strains it is quite definite.

Fuzz, distribution. All grades of fuzziness are found, from naked seed with no hairs other than the lint, to seed entirely covered with fuzz. Intermediate types bear a small tuft at the micropylar end only, or a patch at the base of the seed as well.

These four types are quite definite. Some fluctuation occurs in odd seeds, but examination of a sample of twenty seeds will enable one to assign the strain to its type.

Testa, surface. Smooth, or streaked and corrugated.

This fluctuates greatly, even in the same boll. It is useless for comparative examination. Some strains may be permanently streaked, and some permanently smooth[2], but none such have appeared in the fifty plants which were grown from described seeds for this purpose.

Testa, colour. Black, dark brown, olive green, chestnut, or reddish orange.

The last is only found in immature seeds. Black appears to be definite, but the other three are interchangeable.

Seed shape. Long or round, tip pointed or blunt, tip symmetrical axially or bilaterally.

It is an erratic character which seems to be in part determined by the shape of the embryo, and is not yet worked out.

Stalk or Funiculus. Long or short.

A constant character but easily defaced in storage.

[1] *K. A. S. Yr. Bk.* 1906, No. 89.

[2] The character is employed by Watt, however, as descriptive.

Descriptive characters of seed cotton.

Major length of lint. Nearly always at the chalazal end. The occurrence of occasional hairs of abnormal length[1] may be due to irregular environment, and I have not yet seen it in *combed* Egyptian cottons, nor even in cottons notorious for the character[2], if these are grown in Egypt[3].

The length character is one of great importance, and does not seem to have been studied from the individualistic aspect in pre-Mendelian work. These experiments make it certain that every strain of cotton has the property of forming lint of a definite minimum length. Under certain conditions this minimum may be exceeded, but not by 25 °/₀, even under extreme conditions. These extreme conditions are, in Egypt,

(*a*) Cultivation in the cool north of the Delta[4].

(*b*) Crossing with an entirely different type of cotton[5], such as Uplands.

(*c*) Possibly special manurial supplies.

Not by crossing with botanically allied forms, even when such forms come from the other hemisphere and from a very different climate.

When the first bolls on a plant contain lint which is suspected to be longer than the innate length, this real and unintensified length can be determined in two ways, at present:

(i) By comparative examination of the bolls formed late in the year.

(ii) By examination of the few scanty and fragile hairs which are borne on the unfertilised and aborted seeds frequently found in all bolls.

In these situations the lint-length regresses to the normal.

Distribution of lint hairs on the seed coat. Almost uniform all over; denser at chalazal end; micropylar end naked; whole of micropylar half of seed devoid of lint.

[1] F. Fletcher, this *Journal*, Vol. II. Pt 3.

[2] *E.g.* "Griffin" Upland. See *U. S. Dep. Ag. Bur. Pl. Ind.*, "Distrib. of Seed in 1903," p. 7.

[3] H. A. Allard, *U. S. Dep. Ag. Bur. Pl. Ind. Bull.* CXI. 2, explains these abnormally long hairs by simple knotting.

[4] See also *K. A. S. Yr. Bk.* 1906. [5] p. 361.

The fluctuation of this character is slight. It seems not unlikely that this may be in part correlated with the distribution of the fuzz, at least in the case of seeds devoid of fuzz.

Relative length of the lint hairs on various parts of the seed. Most cottons bear shorter hair at the micropylar end than at the chalazal end, but some are quite uniform. The character is one of much practical importance; a strain bearing irregular lint gives an irregular product even if it be pure.

Fluctuation is slight; a lint pattern can be assigned to each strain[1].

Density of lint hairs. The proportion of epidermal cells which develop into hairs is very variable, as would be expected from the cytology[2]. Late bolls bear thinly covered seeds, and unfertilised seed coats may carry as few as a dozen hairs.

Colour of the lint. May be brown, green, or white. The green is found in certain strains of cotton[3] which occur spasmodically among American Uplands, and appears to be identical with the green of the fuzz, which is much commoner. It may have some interest in connexion with the evolution of the cotton plant. These green cottons fade readily in sunlight to brown, and thence to dingy white. When the green colour is transferred to the finer Egyptian lint by crossing, it fades very rapidly, so that in a single day the boll of green cotton is scarcely distinguishable externally from the recessive white. The relation of this phenomenon to the structure of the lint hair wall has yet to be studied.

Brown cottons also fade in the sun, especially Egyptians, though much more slowly than green.

White cotton can always be distinguished from faded coloured cottons.

Strength, twist, lustre, and fluffiness of lint. These fluctuate very much. They are presumably dependent on the amount, uniformity, and texture of the cellulose deposited on the hair cell wall. These characters are dependent on the method of cultivation[4], provided that the plant is suited to the climate in which it is grown; marked initial differences exist of course between various kinds of cotton[5].

[1] Yves, Henry, "Determination de la valeur commerciale des fibres de Coton." Paris. 1902. [2] *K. A. S. Yr. Bk.* 1905.

[3] "Texas Wool," supplied by D. N. Shoemaker, Esq., Waco, Texas.

[4] Lint born on "rattoons" is always weak.

[5] *E.g.* Sea Island and Short Staple Upland.

Section 2.

Composition of Commercial Varieties.

Once the fluctuation of the seed characters had been determined it was possible to say with certainty whether two cottons were essentially different, or whether they were merely different because of environmental influences.

The examination of the various varieties has been merely a matter of accumulating descriptions which it would be useless to present here. The general conclusions drawn may be stated thus:

1. The Egyptian crop consists of an immense number of different strains (elementary species ?) even when seed characters are considered alone.

2. New varieties show no superiority in this respect over the old ones, viz. Ashmouni, Afifi (1883 abt.), Abbassi (1894 abt.), and Yannovitch (1899 abt.).

3. Every character described in the preceding section can be found in the crop with the exception of green lint.

4. The average length of the lint (major length) is about 30 mm., ranging in a symmetrical curve from 18 mm. to 40 mm.

5. Ashmouni bears shorter lint than the other varieties, Sultani (1906 abt.) rather longer lint.

6. The different varieties showed no obvious differentiating characters, other than the colour of the lint. This is readily understood when we consider that this is the only character which can be recognised without subjecting the seed to a searching examination. The quality of the lint is different in certain varieties, thus brown Yannovitch deprived of its colour in the F2 of a cross with white Upland cotton has been taken for "Sea Island, and not Abbassi."

It is only fair to state, however, that the different varieties are said by agriculturists to require slightly different methods of cultivation. Again, cotton experts inform me that Yannovitch is distinguished from Abbassi by a most curious physiological character, *i.e.* that in the former the value of the second picking lint is not much less than that of the first picking, whereas in the latter the degradation in value is considerable. Such differences are none the less averages, and exceptions from the mean are easily found.

SECTION 3.

Gametic Constitution of the Crop.

The experiment on which the first approximate conclusions were formed was conducted in the following way.

Small samples of seed cotton each containing about eight seeds from a single plant were taken at random from commercial samples of several varieties of the Egyptian cotton crop of 1904. Larger quantities would have been advisable, but they were not obtainable at the time. One hundred of such samples were collected, and described; one seed from each was filed for future reference.

The characters employed in the description were four: maximum length of lint, distribution of fuzz on the seed, regularity of length of lint, and distribution of lint on the seed.

These hundred samples were sown in small plots in 1905. Owing to damage done by the "Sore-shin" fungus, which had not then been recognised, only seventy-five sets survived; of these several were reduced to a single plant, but even with this remnant strong indicative results were obtained, which have since been confirmed in other ways.

It should be understood that at the time when the experiment was made we did not even know if the seed characters were purely maternal, though the cytological evidence indicated such to be the case. Neither had we any clue to the course of dominance, nor even any proof of segregation. The analysis of the results of this experiment gave the first data on these subjects, but it will save time in description if we begin at the wrong end, with the following information:

(*a*) The seed characters are purely maternal, excepting possibly the shape of the seed.

(*b*) The allelomorphs involved are:

1.	Long lint.	Short lint.
2.	More fuzz.	Less fuzz.
3.	Regular length of lint.	Irregular length of lint.
4.	Even distribution of lint.	Uneven distribution of lint.

The dominance is complete in each case, the dominant character being written first. The third and fourth pair are less easy to work with, and are only provisionally accepted as yet. There is a greater perceptible range of difference among Egyptian plants in lint length, than in fuzziness, as the following figures show[1].

[1] Table B.

Experimental results. The seventy-five sets of offspring were examined for their seed characters, and compared with the filed seed and description, with the following results:

A.	Offspring all like parent ...	38
	Offspring all unlike parent ...	37
B.	Cases of alteration in individual characters.	
	Maximum length	25
	Amount of fuzz	15
	Regularity of length	16
	Distribution	14
C.	Cases of alteration in groups of characters.	
	In all four	2
	In three	7
	In two	15
	In one	13

D.	Alteration in maximum staple length.	
	Some or all with shorter lint than the parent ...	21
	All with longer lint than the parent	4
		25
E.	Alterations in seed fuzz.	
	Some or all with less fuzz than the parent ...	12
	All with more fuzz than the parent	3
		15

We will assume that all the ovules of a flower are either naturally selfed or naturally cross-fertilised together. This assumption is not quite true, for I have obtained fertilisation artificially by using very small amounts of the foreign pollen, but it is not far removed from the truth according to present evidence.

Formula. Now we know that for y pairs of characters involved in a cross we obtain in F_2

$$2y \text{ homozygotes from } 4y \text{ individuals.}$$

Thus, with our four pairs we get 1 homozygote in 16 individuals, provided that the parent is self-fertilised. Yet, we find that 50 % of our plants were heterozygotes in these four characters. How much cross-fertilisation takes place each year?

Let P and H represent homo- and heterozygote respectively.

In each generation let xP become H and yH become P.

Then the composition of the crop will be as follows:

1st year: P,

2nd year: $(1-x)P + xH$,

3rd year: $\{(1-x)^2 + xy\}P + \{x(2-x-y)\}H$,

4th year: $\{(1-x)^3 + xy(3-2x-y)\}P + x\{3 - 3(x+y) + (x+y)^2\}H$.

We can now see the law of formation for the H term.

$$\text{In } n \text{ years: } H = \frac{x\{1-(1-\overline{x+y})^{n-1}\}}{x+y}.$$

When "n" is infinite (or practically when more than 10 in the case of our cotton),

$$x\{1-(1-\overline{x+y})^{n-1}\} = x,$$

and

$$H = \frac{x}{x+y}.$$

Example. In our cotton population,

$$H \ldots 50\,\% \text{ or } \tfrac{1}{2},$$
$$y \ldots 1/16.$$

Hence $\quad$ [1] $x \ldots 1/16$ or $6\,\%$.

Thus the amount of cross-fertilisation taking place every year in our crop is about $6\,\%$.

This figure is probably below the truth, on account of the difficulty of recognising alteration in cases where the parents differed but little. We can obtain further evidence from the figures, however, in the following way.

Provided that the annual amount of cross-fertilisation is slight, we can see that the alterations in lint length, for example, will be as follows:

Let L represent long lint, and S represent short lint.

And L is completely dominant over S.

Then, $\quad$ S yields nearly all SS.

Nearly half L yields LL.

Nearly half L yields $LL + 2LS + SS$.

Of the remainder of the population,

$$\left\{\begin{array}{l} \left.\begin{array}{c}\text{When } S \text{ yields any } L\\ \text{or}\\ L \text{ yields } 2LL \div 2SS\end{array}\right\} \text{Cross-fertilisation must have happened in the previous year.}\\ \text{When } L \text{ yields } L \ldots \} \text{ Crossing may have happened in the previous year.}\end{array}\right.$$

[1] Misprinted "y" in *K. A. S. Yr. Bk.* 1906.

Since the chances of S crossing with L, and of L crossing with S are equal, therefore

The total amount of crossing of staple-length characters in the previous year (or "x") is got by doubling the number of cases of S yielding L.

Now apply this to the figures obtained experimentally,

(I) Taking the case of lint length only, we have:

4 cases of short yielding long in 75 plants[1].

Therefore, cross-fertilisation in 1904 was 8 °/₀.

(II) Taking the pair, lint length and seed fuzz, there were:

5 cases of recessives yielding dominants in 75 plants.

26 cases of dominants yielding recessives (12 being heterozygotes in both characters).

Thus, $H = 26/75$ or $1/3$ about,

$$y = \frac{2^2}{4^2} \text{ or } \frac{1}{4}.$$

And
$$\frac{x}{x+y} = 1/3,$$
$$\therefore\ x = 1/8,$$

and
$$\frac{75}{8} = 9{\cdot}4 \text{ or } 12\tfrac{1}{2}\ ^\circ/_\circ.$$

Thus by calculating back the probable amount of cross-fertilisation in 1904 from the existing number of heterozygotes, we obtain the result $12\frac{1}{2}$ °/₀.

Whereas, by actual experiment we found 5×2 or $13\frac{1}{3}$ °/₀.

The above figures are too small to be of real value in themselves, but the approximation obtained has since been shown to be a fair one, by notes on the amount of contamination which isolated strains and F1 plants undergo by vicinism.

The method has been described in detail, although the numbers are small because the application of Mendel's Law in some such way should be of use to all students of genetics who are confronted with a demand for the improvement of a crop which is not entirely self-fertilised[2].

It will be noticed that this conclusion—that all kinds of cotton which I have grown in Egypt are liable to *at least* five per cent. of cross-fertilisation per annum—is diametrically opposed to the opinion

[1] Table D. [2] *E.g.* Sugar, maize and root-crops.

of many writers on cotton. Thus Gammie states his contention to "be that Indian cottons are normally self-fertilised[1]," and quotes Fletcher in support of the view, while Burkill[2] also comes to the conclusion that "insects...in Behar, do produce an effect; but it is an extremely small one indeed—merely a hybrid plant here, and a hybrid plant there," seventeen possible cases in one hundred thousand being the actual figures. It may be that the rule which applies to Egyptian and to Upland cottons does not hold good for the Indian forms[3]. It is also possible that the "variations" due to gametic segregation have not been disentangled from those due to environmental fluctuation; much miscellaneous evidence can be adduced in support of this view, which was certainly held in an extreme form in Egypt[4].

Webber, on the other hand, has shown experimentally that "it would seem that ordinarily only from 5 to 10 per cent. of the seeds are normally cross-fecundated[5]." The objection has been raised to this statement that his results were obtained by study of markedly dissimilar kinds, in order that crossing might be detected easily; this objection is disposed of in the crop analysis described above, for there the seeds were not subject to any isolation nor to experimental treatment, until they were taken from commercial samples to be tested for their gametic composition; the differences between the various strains which were proved to have intercrossed was so slight, moreover, as to be unrecognisable to the agriculturist.

PART II. MENDEL'S LAW IN COTTON.

The cotton plant is not an easy subject for heredity experiments; the plants are large, the flowers are not entirely self-fertilised, and many of the characters fluctuate considerably. Numerous pests attack the experimental plot. Two fungi are important; sooty mould in the late summer, and the "Sore-shin" fungus in the spring. The latter attacks the germinating seed, destroying it, or delaying its growth, but the "Naphthalene dressing" is now available for the control of this plague[6].

[1] *Mem. Dept. Ag. India*, II. 2, p. 2.

[2] *Journ. and Proc. Asiatic Soc. Bengal*, New Ser. Vol. III. No. 7, 1907, p. 524.

[3] Leake, *loc. cit.* proves conclusively by study of leaf characters that the speculations about the self-fertilisation of Indian cottons were without foundation.

[4] See p. 347, on the nature of Hindi.

[5] *Yr. Bk. U. S. Dep. Ag.* 1902, p. 370.

[6] *K. A. S. Yr. Bk.* 1906.

The chief insect pests are Aphides, Boll worm, and the cotton Stainer Bug. The two latter are practically uncontrollable.

The rapid response of the growing plant to changes in the limiting factor of the environment makes it advisable to work with statistical descriptions of individuals when such a method is possible; at present this has not been done to any great extent, but with a residential experiment station much more use will be made of statistical methods, in order to study such characters as habit of flowering, time of maturity, etc.

One great advantage of the cotton plant is that it can be grown on as a perennial by simply cutting it back and allowing it to shoot from the base in the following year; thus, if more seed is required, or protected seed in place of natural seed, it can readily be obtained year after year.

There are three points of the utmost importance to be borne in mind when experimenting on genetics with cotton. These three points belong by right to the conclusion of this paper, since they are deductions from the results described in it, but it is well to recognise their importance at the outset.

These points are:

1. No pedigree can be considered exact, unless the direct ancestors have been all self-fertilised. Even five per cent. cross-fertilisation per annum will entirely vitiate the statistical expressions of Mendel's Law in the F3. When, as in pre-Mendelian times, selection was carried on in mass-cultures for as many as seven years, the chances of a plant representing its name at the end of that time were probably even! Absolute freedom from vicinism is perhaps impossible on a large scale, but the use of netting[1] abolishes it when netting is feasible.

2. The desirable characters are mostly dominant over the undesirable ones[2], except in cases where the heterozygote is intermediate. This makes deliberate synthesis of commercially useful cottons more tedious than it might otherwise be.

3. It must be remembered that the parent plants used in a cross are almost certain to be heterozygotes in some characters, for the conclusions drawn in the first section of this paper appear to apply equally to all the kinds of cotton which I have yet examined. Consequently, the descendants of each F1 plant should be kept separate from those of the others.

[1] p. 359. [2] p. 362.

The point might seem obvious, but it does not appear to have been fully realised by some workers who are dealing with hybrid crops.

4. One is tempted to add that the intensification by crossing of various characters (such as lint length[1]) is likely to give rise to unfounded expectations on the part of those who are doing synthetical work, but there is some evidence from other organisms to show that this intensification may perhaps be permanent.

Methods. The keeping of records of all kinds, taken at all stages of growth, and all hours of the day, in addition to laboratory examination of the seed, is necessitating the development of special card-filing systems in preference to the use of note-books.

Seeds which have been combed out for examination are also stored for reference in card-files, by covering them with squares of celluloid, the corners of which are inserted in slots in the black cards. An identification label is provided, and descriptions can be written on the white back of the card.

Stout paper bags are employed for storing small samples of lint and seed cotton. These are filed vertically with guide cards in sets of drawers made on the "elastic" system, so that the drawers can be taken into the field and filled with the samples directly. Much sorting of samples is obviated by this plan.

The most important details of method are those dealing with the exclusion of bees by nets, and the prevention of "Sore-shin." The latter has been referred to elsewhere, and it is only necessary to point out the immense importance of such prevention, in that it enables us to raise many more seedlings from a limited quantity of seed.

The netting of plants is at present done with mosquito nets, each two metres high and one metre square, the top being made of stout calico. These nets are supported on two-inch-square sticks, which are placed inside the net, one at each corner. The upper end of each stick is covered with a rough pad of rags to prevent chafing of the calico; the lower ends are surrounded at the ground level by four thin boards, measuring 100 cm. by 30 cm., placed on edge, and the foot of the net is rolled up and held against these boards by an encircling cord. Unless these foot-boards are used the net rots at the soil level. We estimate that with care, and occasional spraying, these nets should last three years apiece. They cost about ten shillings each, but they are as

[1] p. 361, etc.

essential to the conduct of the experiments as the cotton plants themselves.

For propagation of pure strains on the Central Station it will probably be found cheaper to build large cages of fine wire net, each holding some fifty plants. The seed from these will be sent out to the sub-stations for propagation.

The cross-pollination of the cotton flower appears to be effected almost entirely by bees. Many insects are to be found in the flowers, and a list is being prepared by Mr Willcocks[1], but all those which are capable of bearing foreign pollen[2] are excluded by the nets; there does not seem to be any appreciable amount of wind-fertilisation, and even wind-borne pollen would probably be arrested by them.

Before the nets were adopted the preparation of self-fertilised seed was laborious and uncertain. Tissue paper bags were employed for separate flowers, and a large proportion of the bagged flowers was shed afterwards, probably on account of interference with transpiration.

The operation of crossing has also been simplified by the nets. The flowers are castrated at 4 a.m., and cross-pollinated at 9 a.m. No bags are necessary if the other open flowers under the net are removed. The flower from the plant which is to be employed as the male parent is, of course, covered with a bag of paper.

The First Generation.

The details concerning dominance will be described under the next heading.

The crosses on which the results were obtained were as follows: the common names alone are given, which will enable those acquainted with cotton to recognise the types dealt with, while details as to characters will be gathered from the second generation descriptions. It seems somewhat useless to attempt to give rigid names to hybrid plants, such as the Afifi parent in 252.

Crosses in Second Generation, 1907.

250. Texas Wool (Upland) × Abbassi (Egyptian).

251. Afifi (Egyptian) × Natural hybrid of Hindi and Egyptian.

252. Afifi (taken from Brown Yannovitch) × Truitt Big Boll (Upland).

[1] Entomologist to the *K. A. S.*

[2] See also Burkill, *loc. cit.*; H. Müller, *The Fertilization of Flowers*, p. 145; W. Trelease, *Nectar*, Washington, 1879; Toniet, *Histoire des Drogues*, Paris, 1694, Plate of Cotton plant with insects flying.

Cross in First Generation, 1907.

253. Hindi (Egyptian weed cotton) × Charara (Egyptian).

254. Charara × Wild cotton of Moqui Indians from Arizona.

In no case were all the plants of the F1 identical. The existence of heterozygotes in one or both parents was shown in the following characters, by one or another.

Seed fuzz, length of lint, spot of leaf, shape of leaf, habit of growth, colour of corolla, colour of anthers, and seasonal formation of flowers.

The most striking feature is the intensification of certain characters which results when two botanically dissimilar cottons are crossed together. This intensification is shown in the height of the plant, the time of flowering[1], the length of the lint, the size of the seed, probably in the fuzziness of the seed, etc. Of these the length of the lint has been observed most fully.

In cross 252 a 30 mm. cotton was crossed with a 20 mm. cotton. The 30 mm. one was known to be heterozygous in length of lint, because its offspring had comprised both 30 mm. and 24 mm. cottons. We should have expected that the F1 would consist of equal numbers of 30 mm. and 24 mm. cottons, both being heterozygotes containing the recessive 20 mm. form.

Such was not the case, for when the three F1 plants ripened, two were found to bear 30 mm. lint, while the lint of the other was 35 mm. long. By comparison of this series with another one (250), where the parent's lint had been respectively 25 mm. and 23 mm. long, while the F1 bore 30 mm. lint, it was clear that the two allelomorphs of the heterozygote parent in the former series had been separately acted on by their union with the American 20 mm. gametes in such manner as to stimulate them; the result of this stimulation (or possibly reconstruction) being that the character which they controlled was hypertrophied.

That the character is essentially unchanged may be seen by examination of the lint born in the late bolls at the beginning of the winter, or else of the few lint hairs born on those unfertilised seeds which are found in all bolls. In these two situations the major length of the lint is found to be the same as that of the parent; the 35 mm. F1 drops to 30 mm. and the 30 mm. F1 drops to 24 mm. This shows that the intensification is connected with nutrition; not with temperature, except in so far as the temperature may limit the food-supply. This

[1] In cross 254:—1st flower in parent-strains, and F1, appeared on following dates: *Hindi*, 1st three plants, June 11th, 15th, 30th; *Charara*, 1st four plants, June 12th, 13th, 14th, 14th; F1, three plants, June 4th, 5th, 6th.

conclusion is supported by the fact that the same intensification in a slighter degree can be effected on the Egyptian parent by growing it in the north of Egypt.

In contrast to this we have the series 253 in which practically no intensification of length was found in F1.

This intensification on crossing is a well-known phenomenon. Many of the old examples are not cases of intensification proper, but omitting these we have still a number of cases which are parallel to ours. The question now arises as to the stability of such intensified characters in subsequent generations. Some results obtained by Biffen[1] on intensification of the length of the internodes of wheat ears indicate that the intensification may be permanent in some strains[2]. If this is so for cotton we may hope, contrary to my first expectation, not merely to combine existing characters into one strain, but even to make new characters. In any case, this is by far the most interesting and important problem offered for our study by the cotton plant.

THE SECOND GENERATION.

The evidence at present available is based on naturally fertilised seed, with a probable error of 10 °/₀. The result of this is that hardly any of the existing F3 embryos will be grown, and next year will be devoted to a thorough examination of four large sets of F2 plants grown from netted plants. Some experience has been obtained this year in the methods of obtaining and treating statistics for the individual, and these methods will be applied on a large scale.

Provisional List of Allelomorphic Pairs.

Complete dominance.

*Glabrous petiole in seedling.	Hirsute petiole.
Light green leaf.	Dark green leaf.
Long filaments of anthers.	Short filaments.
Long style (?).	Short style (?).
Large seed.	Small seed.
*More fuzz on seed.	Less fuzz.
*Coloured fuzz.	White fuzz.
*Long lint.	Short lint.
Regular length of lint.	Irregular length.
Even distribution of lint.	Uneven distribution.
*Egyptian quality of lint.	Upland quality.

[1] Unpublished.

[2] See however, Johanssen, "Int. Conf. on Plant Genetics," *R. Hort. Soc. Jour.* 1907.

Heterozygote intermediate.

*Red spot of leaf.	Faint spot.
*Yellow petal.	Cream (white) petal.
*Large purple spot on petal.	No spot.
*Rich yellow anthers.	Buff anthers.
First stem-branches flowering.	Branches vegetative.
Tall stem.	Short stem.
Early flowering.	Late flowering.
Early cessation of flowering.	Continuous flowers till December.
Early maturity.	Late maturity.
Coloured lint.	White lint.

* Asterisks denote reliable evidence.

This list is compiled from two sources. Firstly, the pedigrees of some of the natural hybrids referred to in the first section of this paper; secondly, from the examination of the F2 of the following:

252 A	... ... 56 plants.	252 B	... ... 30 plants.
252 C	... ... 30 plants.	250 A—G	... 75 plants.

The comparison of the offspring of the three sister plants, 252 A, B, and C, has enabled many points to be seen which must otherwise have remained obscure; because, on account of the unpleasant conditions under which the work has been carried on up to the present, it has not been possible to obtain sufficient data about the parents which were employed.

The characters will be discussed in the order in which they appear during the growth of the plant. Numbers obtained are aggregated when all the sets are comparable; plants stunted by "Sore-shin" are omitted when dealing with physiological characters; hence the irregular totals.

Red spot of leaf. The character is due to the development of anthocyan in the epidermal and sub-epidermal cells of the petiole at the point where it divides into the leaf-veins. The colouration of the "red-leaf" Upland cottons[1] is due to further development of the same character.

Like all anthocyan colours it is dependent on the illumination, and also on temperature, and water supply. Under similar conditions of cultivation the types are sharply distinguished.

Crosses of spotted with spotless give spotted F1, but the intensity of the colour is less than in the spotted parent. The heterozygote spot

[1] *E.g.* "Willett's Red Leaf."

appears to be distinguishable from the homozygote spot in F2. The following count was obtained on all seedlings:

Spot (homo- and heterozygote) ...	187
No spot	65

The character may be regarded as a capability of reaction to *light*. It is also of practical importance in connexion with the eradication of the weed-cotton called "Hindi" from the Egyptian crop, since the former has a full red spot on the leaf, whereas the great majority of the Egyptian plants have only a faint spot. The F1 of natural hybrids between Hindi and Egyptian can be detected better by this character than by any other.

Hairiness. The petiole of the Egyptian parents bears a few hairs on the dorsal side, and a very few sparse hairs on the ventral side; the petiole of the Upland parents is hirsute all round, though more densely so on the dorsal side.

Petiole of the F1 seedling resembles the glabrous parent.

The following count in F2 was obtained:

Glabrous	... 111	Hirsute	... 37

This expression of the case does not appear to cover the whole ground, for the hairiness of the leaf surface and stem appears to be separate factors, possibly correlated with the simple one described.

Leaves. The leaf-characters are the hardest of all to judge, on account of the many factors which appear to make up the leaf shape; a good deal of fluctuation has also to be eliminated in the early stages of the plant's growth[1], and some plants appear to form mature leaves at an earlier stage than others. They will be more easily studied in the F3.

The form of the leaf would not appear at first sight to be of much practical importance, but the sensitiveness of the "lint-quality" is such that the shape and size of the leaves may be able to affect it.

In the series 252 the Upland parent had a lighter green leaf than the Egyptian. F1 was intermediate, and dark green plants were recognisable in F2:

Light green	... 93	Dark green	... 28

[1] T. H. Middleton, *Agric. Ledger*, 1896.

In the matter of other leaf characters the following analysis seems to be indicated[1]:

(*a*)	Large lamina.	Intermediate lamina.	Small lamina.
(*b*)	Slightly dissected.	Deeply dissected.	Very deeply dissected.
(*c*)	Broad segments.	Medium segments.	Narrow segments.
(*d*)	Shape of tip of segments.		
(*e*)	Flatness of lamina.		
(*f*)	Thickness of leaf.		
(*g*)	Hirsuteness.		

Height of stem. The height of the plant is intensified in F1 to a considerable extent, perhaps 50 °/$_o$. As we do not know to what this intensification is due we should hardly expect to find conspicuous segregation in F2.

Curves of height were plotted for both series 250 and 252 at the end of June and again at the end of July. The results show very little, but indicate that segregation has occurred, together with intensification.

Series 250. Both parents of about the same height, *e.g.* on a given date about 60 cm.

F1 on same date would have been perhaps 100 cm. high.

F2 curve of heights on same date ranged from 30 cm. to 100 cm., although the plants had been practically level till two months old.

Series 252. The Egyptian parent was very tall, and bred pure to the character of tallness through three generations, being one of the tall plants which are common in the Egyptian cotton fields, and are by some supposed to represent the remains of the old variety "Hamouli."

The F1 plants were all taller still, when growing side by side with the parents in 1906.

F2 curve of heights again showed a range which exceeded that of either parent, with indications of three crests, corresponding to 1 : 2 : 1.

The subject will be investigated next year by taking measurements every fortnight, through a large population. The growth-time curve of the individuals is likely to show important results.

Habit of growth. The form of the stem is subject to some fluctuation. There seems to be at least one character concerned which cannot be obliterated, and provides an allelomorphic pair.

[1] See however, Leake, *loc. cit.*, who has given us a statistical expression for some of these characters.

The distinction within this pair lies in the nature of the first branches produced by the seedling plant; these may be monopodial and vegetative, or sympodial and flowering directly. In the former case the stem branches at the base, while in the latter the stem grows straight up, putting out only flowering branches. There also seems to be a second character which may be superimposed on this, namely, whether basal branches are put out late in the summer or not, as we shall see shortly.

We shall refer to the form in which flowering branches alone are formed at first as "unbranched" from the vegetative point of view, and to the other as "branched."

The cross of these two types (of which the latter is the usual one among Egyptians) gives a kind of intermediate in F1, in that it sends up a leading shoot with flowering branches, but quickly surrounds this at the base with laterals.

In the F2 we had the following figures in the middle of June:

250 A, G.	Branched	... 58	252 A.	Branched	... 21
	Unbranched	... 22		Unbranched	... 27
252 B.	Branched	... 18	252 C.	Branched	... 18
	Unbranched	... 8		Unbranched	... 6

On this date, it will be noticed, while 250, 252 B, and 252 C gave a plain 3 : 1 ratio, the ratio in 252 A was 7 : 9. This might be taken as an indication (and nothing more) of the existence of a second pair of allelomorphs interacting on the first pair. Further probability is given to this idea by a later count made at the end of July, when the so-called "second growth" has begun in the field crop.

The result of this second count was:

250 A, G.	All branched.	252 A.	Branched	... 35
252 B.	Do.		Unbranched	... 13
252 C.	Do.			

Now we know that the Egyptian parent in 252 was a hybrid in respect of length of lint, and we shall see shortly that the curves of flower-production are entirely different in the 252 A series from those in the series 252 B and C, so it is by no means unlikely that it was also hybrid in respect of habit, although pure tall. The Upland parent gave uniform "unbranched" descendants.

There would seem to be a cryptomere present, but it would appear that the "cryptomere" is only such in the early stages of growth. However rash this speculation may appear, it is certain that the differences between these series were not merely accidental.

Flower colour. The flower colour characters have shown a pleasant simplicity by comparison with the preceding characters, and have not yet fulfilled my hopes of complexity[1].

The units involved and the heterozygote forms are:

Homozygote.	*Heterozygote.*	*Homozygote.*
Yellow petal.	Lemon petal.	Cream (white) petal.
Large purple spot.	Small purple spot.	No spot.
Rich yellow anthers.	Pale yellow anthers.	Buff anthers.

Fletcher[2] has shown also that entirely red petal is dominant over yellow petal, which agrees with the behaviour of the partially red (spotted) petal. His red parent seems to have been a heterozygote containing yellow.

These colours are all due to the sap, plastids being devoid of colour.

Series 250. The Egyptian parent had a very small spot, which was nearly extinguished by fluctuation in some flowers. The character was presumably pure, giving no other form in the ten descendants which were grown. The excess of spotless forms shown in the F2 is certainly due to increased fluctuation, for it was common to record a plant once or twice as spotless, and then find a flower appearing which bore a faint spot on one or more petals. The same was true of the F1. The counts in F2 gave the following figures, each of the seven strains showing all three forms:

Petal: Yellow	... 16	Lemon	... 36	White	... 17
Spot: Spot full	... 5	Spot faint	... 9	No spot	... 47
Anthers: Yellow or pale yellow		...	... 47	Buff	... 22

Series 252. Here the Egyptian parent had a full and conspicuous purple spot, which could not be extinguished by fluctuation.

Petal: Yellow	... 21	Lemon	... 50	White	... 23
Spot: Spot full	... 23	Spot slight	... 42	No spot	... 31
Anthers: Yellow or pale yellow		...	... 72	Buff	... 22

A partial count of the yellow to pale yellow anthers gave 13 : 28. These new colour combinations are very handsome in some cases, and might be turned to practical account in providing identification marks for new strains of cotton. One point worth further attention was noted in working out the ratios of the various combinations of colours; it might be due to vicinism, or to partial gametic coupling, but instead of getting the expected ratios of the various combinations we find

[1] This *Journal, loc. cit.* [2] *loc. cit.*

an excess of what we may call "positive colouration" at one end of the scale, and of "negative colouration" at the other. The matter will receive attention next year, and the preliminary facts given here may be of interest to other workers.

In series 252 there were 94 plants. The expectation on 96 plants is placed in brackets after each figure.

	Full spot.	Spot slight.	No spot.	Total.
Yellow petal ...	11 (6)	5 (12)	2 (6)	18 (24)
Lemon petal ...	9 (12)	27 (24)	17 (12)	53 (48)
White petal ...	3 (6)	8 (12)	12 (6)	23 (24)

	Yellow petal.	Lemon petal.	White petal.	Total.
Yellow anthers...	17 (18)	43 (36)	12 (18)	72 (72)
Buff anthers ...	1 (6)	10 (12)	11 (16)	22 (24)

	Spot full.	Spot slight.	No spot.	Total.
Yellow anthers...	23 (18)	31 (36)	18 (18)	72 (72)
Buff anthers ...	0 (6)	9 (12)	13 (6)	22 (24)

My expectation that the colour inheritance might be complex was based on the fact that these colours are all closely related substances, thus, the spot character might have been manifested only when the morphological and chemical factors required for its formation had met in zygote formation. This is not the case, but these figures seem to indicate possible partial coupling.

With regard to the occurrence of 2·5 °/₀ of red flowers among the descendants of yellow recessives, described by Fletcher[1], it would seem very probable that this was due simply to vicinism. There seems to be no reason why vicinism should be of less importance in India than in Egypt[2], and the percentage obtained by Fletcher comes well within the Egyptian average, even if the number of red flowers in the vicinity was much less than the number of yellow flowers.

Other flower characters. Counts have been made of the length of the filaments, column, and style. The two latter characters fluctuate too much to be distinguished, unless statistical methods can be used. Long filaments appear to segregate from short, the former being dominant.

These characters are worth attention in one respect: the combination of long filaments and column with short style should be a very difficult flower for any insect to cross-fertilise. It might be possible, perhaps, to synthesise in this way a strain of cotton which would be practically cleistogamic.

[1] *loc. cit.* [2] Confirmed by Leake, *loc. cit.*

Tubular corollas appear to segregate from the campanulate corolla found in Upland cottons.

Calyx characters also fluctuate considerably, and will have to be followed out in later generations.

Flower formation. The immensely important question of the time of maturity is connected with this character.

In studying flower formation all plants which have been stunted by "Sore-shin" are disregarded, such plants having been noted in the seedling stage. Habit of growth has also been taken into account, but the only difference which it appears to make in this respect is that the "unbranched" plants are a few days earlier than the rest on the average; this is seen when the curve of the date of the first flower is plotted separately for both habits.

The plot was examined every day from June 3rd, 1907, to the present date (December 31st), and a note made every day of the plants which were in flower. The data thus obtained have been plotted into curves for the individual plants, as well as into other curves, and the results are so interesting that the method will be applied to at least a thousand plants next year, in spite of the labour it involves.

Miscellaneous observations on the behaviour of foreign cottons in Egypt, and of Egyptian cottons elsewhere, have given a strong probability to the idea that each strain of cotton has a definite relationship to temperature in regard to flower-production. Such observations are familiar to all who have grown foreign cottons (if taking precautions to exclude hybridisation); supposing such relationship to exist, it can be more readily seen in Egypt than in any other cotton country, on account of the regular way in which the weather changes as the year goes on. In series 252 the Upland parent was, as usual, earlier in flowering and maturity than the Egyptian.

The results obtained were first plotted to show the date of the first flower, of the average of the first two, and of the average of the first three. The result was practically the same, which ever method was employed, and the first was finally adopted. The curve for the 252 A series appears to indicate that the range is beyond that of either parent, as in the case of height, and the grouping of the dates shows at least an indication of 1 : 2 : 1 ratio.

The curves for 252 B and C were quite similar in form, but the late portion in 252 B was moved up about ten days later. This looks like another character in which the one parent was heterozygote.

The "pure lateness" or "pure earliness" of the two extremes of the curve can only be settled in F3. Meanwhile we have some more results of no little interest.

When the total number of flowers produced on each day of the year was averaged in ten-day periods, plotted separately for 252 A, B, and C, and aggregated for 250 A...G, the curves given in Fig. 1 were obtained. It will be seen that 250 and 252 A give a double-crested curve which is quite distinct from the single crest given by 252 B and C. To ascertain the meaning of this difference we have to determine the form of the curve produced by the individual plants.

Seasonal Production of Flowers.

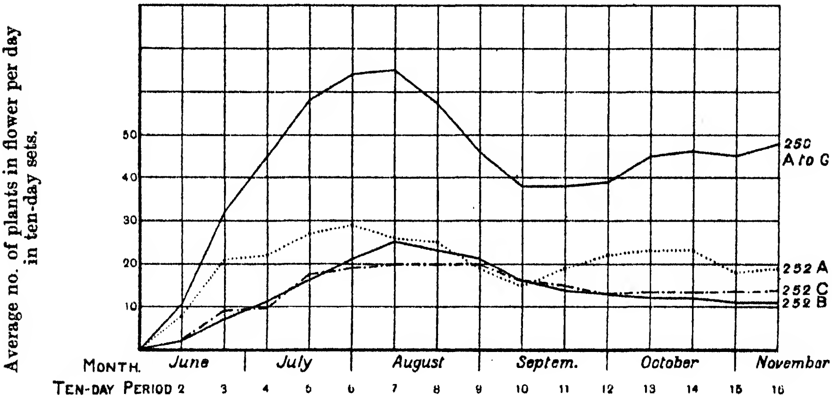


FIG. 1.

When the curve of flower-production in time is plotted for each plant we find some remarkable differences. The general rule which governs these differences has not yet been seen, and it would take too much paper to reproduce every curve. Four types exist:

I. Flowers cease in September. A few appear in December.

II. Flowers cease in September. Flowering resumed in October.

III. Flowers decrease in September and increase again till December.

IV. Flowers continue steadily through December.

The types II. and III. are less common in 252 B and C than in 252 A.

Comparison with the records of other characters shows no connexion of these types with either habit or morphological characters, or even with the date of appearance of the first flowers. The only character common

to any class is that the plants included in I. were marked off in the beginning of September as "early death"; at that time they were rapidly maturing all their bolls and seemed to have ceased work for the year. For the past three years it has been noticed that Uplands, Hindi, etc., start a kind of second growth in the winter, and bear abundant flower buds, but this analysis of an F2 has given the character a definite expression. The character is one of no small importance from the practical point of view, and will have to be worked out with the assistance of a cotton expert to determine the effects on the lint[1].

There are also great differences among the various plants in respect of the number of days on which flowers are produced; some flower very freely, producing many flowers every day, like the F1; while others only flower occasionally. This does not seem to affect the form of the curves of flowering, but only their height. Both slow and rapid flowering are found in all four classes.

Boll characters. These have not yet been investigated in detail. The shape, size, number of divisions, and the appearance of the glands seem to be the four main characters. They will be worked out more easily on the cross of Russell Upland with Charara, since the bolls in the former are very large.

Weight of first picking in 252 *series.* All seed cotton ripe on August 12th was picked off and stored. A second picking was taken on September 7th. These pickings were weighed separately for each plant, and the results show some trace of regularity.

The only correlation which could be found between the figures obtained and the other data concerning such points as the flowering curve, date of first flower, length of lint, height of plant, etc., was that all plants which produced more than ten grams of seed cotton by August 12th belonged to the group which bore the first flower before June 20th, while those which had not produced any seed cotton by September 7th belonged to the group which produced no flowers until after June 30th. The date of production of the first flower may thus be used as an index to the time of maturity.

It is singular that the curve of weight of the pickings shows a small node in 252 A, B, and C also. This node is composed of a small number of plants which bear distinctly more than the rest, and its appearance in all three sets leads one to think that its presence may not be due to mere accident[2].

[1] See p. 375.

[2] Possibly due to conjunction of large boll.

It is also of interest to note that three plants of the five which were known to be vicinists gave figures for weight of seed cotton which were entirely removed from the rest.

Seed Characters.

Weight of seed. The Upland parent in series 252 had a much larger seed than the Egyptian parent. The seed of the F1 was heavier than either parent, and in F2 the curve reproduced here was obtained (Fig. 2). The weights were determined by counting the number of seeds in five grams of ginned seed from the first picking. Small seed segregates from large seed, and the segregated types show a greater range of fluctuation than is shown by either parent.

Weight of Seed.

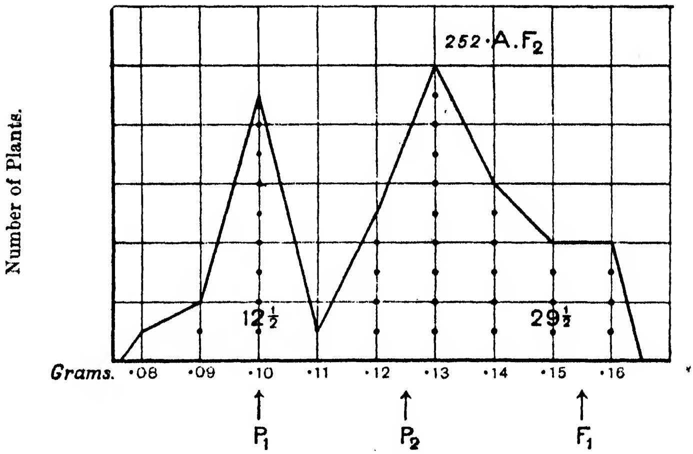


Fig. 2.

Fuzz of seed. The inheritance of this character in cases where the two parents both bore small amounts of fuzz has been followed out in several natural hybrids, and appears to be quite simple, the type with more fuzz being dominant over the type with less fuzz.

In the series 250 and 252 the matter appears to be complicated, but the fact that the F1 was naturally fertilised prevents a definite statement. The slight fuzz of the Egyptian parent was completely dominated in F1 by the entire fuzz of the Upland parent. The slightly fuzzy seed reappeared in F2, but in very small proportions, only 6 in 103 plants of

252 F2; eight others were "semi-fuzzy." Whether this implies the existence of two allelomorphic pairs[1] in crosses where entire fuzz is involved (as is probable) or whether it is due to vicinism, is a matter of considerable importance in constructive work.

It is of interest to note that in the 250 series the Egyptian parent was a natural hybrid made in 1904 between a plant with naked seed and a plant with slight fuzz on the seed; seven F1 plants were raised from a cross of this heterozygote with Upland bearing entire fuzz, and a small F2 raised from each of them. In four the slightly fuzzy type split out, while in the remaining three the naked type recurred; the expectation being of course two to two. The history of this slightly fuzzy strain, derived in 1904 from an unknown pollen parent, dominating the naked seed in 1905, throwing pure recessive naked in 1906, hidden under the entire fuzz of the Upland in 250 F1, and reappearing as a recessive in its turn in 1907, is an excellent example of the grip which the segregation law gives us on the problems of cotton breeding.

Colour of fuzz. The colour is independent of the amount of fuzz. Green fuzz is incompletely dominant over white and brown, but as the latter can be derived from the fading of green, the character will have to be worked out with more suitable material, probably by the use of seed dissected from unripe bolls[2].

Colour of lint. Brown lint (or green) is not completely dominant over paler or white lint, but gives an intermediate heterozygote form. The series 252 A, *e.g.*, gave in F2 the following figures:

Brown	... 12	Cream	... 21	White	... 11

The distinctions in colour cannot be seen well in seed cotton, unless—as in this series—the difference is very marked. This accounts for the conclusion formerly stated that dominance was complete[3]. It is needless to say that the power to differentiate the homozygote without growing its offspring will save much trouble in constructive work.

Length of lint. Long lint is completely dominant over short lint, with intensification of the dominant character in some cases. In F2 the recessive short reappears, and intensification survives partially.

The difference between the parents in series 252 is not sufficient to differentiate the two forms of long and short, since the intensified short

[1] Series 253 gave "reversion" to entirely fuzzy seed in F1!

[2] I have since found by accident that the fuzzy colours are quite distinct when the seed is inspected in bulk from about five metres away.

[3] *K. A. S. Yr. Bk.* 1906.

may be as long as the unintensified long; the curve of lint length given for 252 A F2 (Fig. 3) shows that many of the plants have regressed to the normal, and the question arises as to whether this will continue to happen in successive generations, or whether some strains will retain the intensified length. The long and short forms can be distinguished in the overlap of the curves by comparative examination of the lint formed in the latter part of the growing season, but the methed is tedious; mere examination of the very last bolls is not sufficient, for with some plants the intensification reappears in December.

Length of Lint.

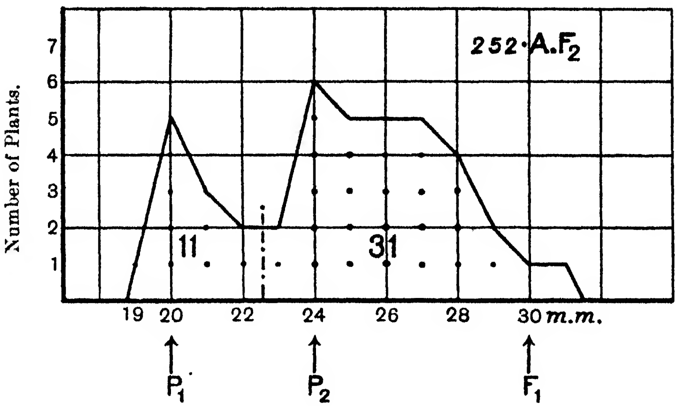


FIG. 3.

There is a possibility that this intensification of length can only be manifested by lint of Egyptian quality (as distinct from Upland). The lengths of the Upland qualities which segregated out in this F2 (see "Quality of Lint") were as follows:

20 mm....3 21 mm....2 23 mm....2 24 mm....2 25 mm....1

These divergences from 20 mm. and 24 mm. are within the error of measurement, and contrast strongly with the fluctuation of all the Egyptian qualities of this generation.

Regularity and uniformity of the lint. The irregular character reappears in F2, but it would be better to wait for the F3 before making a definite statement, as it is not an easy character to judge fairly when the length of lint, fuzz of seed, size of seed, etc., are mixed up with it; the same difficulty holds in examination for uniformity of distribution.

Quality of Lint.

Description of samples of lint from Series 252, by E. A. Benachi, Esq.

Parents

45 A. American quality. White short staple, not equal to any Egyptian cotton.

77 A1. Brown in colour, long staple, Afifi style. Not finer than Tanta Afifi. Medium strength.

77 A2. Afifi.

77 B2. Afifi style and colour.

First generation.

252 A. Similar to full quality and colour of Yannovitch[1].

Second generation (252 A1, etc.).

1. Yannovitch colour but short staple.
3. Light creamy colour, poor staple and weak.
4. Light creamy colour, Yannovitch staple and finish, but not so long.
5. Creamy Yannovitch colour, but staple of poor Afifi.
6. Light colour like Yannovitch, but quality like fine staple of Afifi, and not strong.
9. White, but not dead white. Finer than Hindi. Not desirable cotton.
10. Afifi colour, poor staple, short.
11. White, inferior to Abbassi.
12. Good dark brown colour, but very short staple, not Egyptian.
13. Good brown colour, good staple and strong. (Good all round cotton, like the early Afifi of years ago.)
14. Afifi colour, but staple more short and irregular than any Egyptian cotton, though fineish.
15. White cotton, Abbassi colour but not as long. Finer staple than Abbassi.
16. White, but not dead white colour. Short staple, undesirable cotton like the Hindi.
17. Creamy. Inferior to Yannovitch in staple and fineness.
18. Rather light brown. Good quality of Afifi, regular and good staple.
19. Afifi brown colour. Good staple, regular lint and strong, like good style of Afifi.
20. Creamy but short staple, American style.
21. White, not equal to good Egyptian Afifi, shorter staple.

[1] *I.e.* Creamy, finer and more silky than Afifi.

22. Light creamy colour, but staple like Afifi.
23. Creamy colour like Yannovitch, and finer but not strong staple.
24. White, finer than Abbassi, good staple.
25. Light cream Yannovitch colour, long staple and strong. Better than Yannovitch quality.
26. Good brown colour Afifi, good staple and regular, but not fine enough.
27. Fine Yannovitch staple and colour, strong but not very long. Consider it an improvement on Yannovitch quality.
29. Creamy, inferior to Yannovitch in staple and fineness.
30. Light creamy colour, short, American style.
31. Good brown colour, but not Egyptian quality.
34. Whitish, inferior to Abbassi.
37. White, finer than Abbassi, not up in staple or strength.
38. Creamy, but staple short, inferior to Afifi.
39. Creamy colour like Yannovitch, but finer staple and shorter than Yannovitch.
40. White, inferior to Abbassi.
41. Creamy colour, staple short, not like Egyptian.
42. Creamy, short but fineish. American style.
44. Creamy. Yannovitch style. Good staple, not strong.
43. Brown colour, short and wasty. Staple weak.
45. Creamy, but like short staple Afifi.
46. Creamy, very good staple and strong. Better than Yannovitch. (Strongly recommended.)
48. White, but short staple, inferior to Abbassi.
50. Brown colour, good long staple and fine. Fairly strong. (Good cotton.)
51. White colour, strong and fine silky staple, not very long.
52. Abbassi white, not as long, but finer and strong staple.
53. Brown, but Upland staple.
54. Creamy, but short. Inferior to Yannovitch.

The above criticisms were written by Mr Benachi on the hand-ginned samples of first picking lint from the F2 of 252 A. The only special request made was that as colour would be no criterion of the nature of the cotton it would be advisable to denote the quality both with and without the colour.

Perhaps the most remarkable point about the whole list is the comment on No. 13. This plant is not at all like an Egyptian in

appearance, for it has a large dark green leaf, white flower with heterozygote purple spot, heterozygote stamens, flowered on June 11th, and so forth; it has a curve of flower formation which belongs to Class I., and this was apparently the type of flowering curve of the original Afifi strain; by simple examination of the lint from an unknown plant, Mr Benachi has detected this "early death" character!

Tabulation of this list gives the following figures:

(A) White ... 11 (11) Cream ... 21 (22) Brown ... 12 (11)

(B) Other than Egyptian... 10 (11) Egyptians ... 30 (33)

Not defined ... 4

It is thus clear that we may use American Upland parents in synthetical work, for their desirable qualities, and get rid of the undesirable recessive lint character in the F2.

As regards the classification of the Egyptian qualities inside the group, the figures are as follows:

Afifi...	... 7	Finer than Afifi...	... 1
Yannovitch...	2	„ „ Yannovitch...	5
		„ „ Abbassi	... 7

Inferior to Afifi ...	... 4
„ „ Yannovitch	... 5
„ „ Abbassi	... 2

The probable explanation of these classes is that intensification takes place in the silkiness of the lint as in other characters, so that an extra silky Afifi—as in the F1—is equal to Yannovitch. The matter requires much more investigation, but it would seem to be fairly simple. This "mock Yannovitch" will probably provide a companion example to the "unfixable" Andalusian fowl.

In conclusion I wish to express my sincere thanks to my chief, Mr G. P. Foaden, for his advice and information on agricultural and commercial matters relating to Egyptian cotton, and also for the support which he gave to the Experiment Station project.

To Mr J. R. Gibson, Commissioner of the State Domains, I am indebted for many valuable discussions on cotton problems, and to Mr E. A. Benachi for the examination of the second generation lint samples.

My colleagues in the Society have assisted me loyally with their special knowledge of cognate subjects, and various friends and corre-

spondents in all parts of the world have been most kind in supplying information and seed.

General Conclusions.

1. (*a*) Mendel's Law of Heredity applies to all those characters of the cotton plant which have been critically investigated.

(*b*) Among the allelomorphs concerning which we can as yet advance only provisional hypotheses we find several physiological characters which will enable us to give a definite statistical expression to certain agricultural peculiarities.

2. (*a*) No cases of coupling have yet been discovered which are likely to prevent the synthesis of the desired forms of cotton.

(*b*) The physiological relationships of the plant to the limiting factors of the environment are most important characters from the agricultural point of view, on account of their probable effect on the quality of the lint.

3. (*a*) Most of the characters of the cotton plant fluctuate to some extent, but this fluctuation is far less than has been commonly assumed to be the case.

(*b*) The maximum length of the lint may be forced above the normal by special physiological conditions (either of the environment or of the individual), but it cannot be depressed below that normal.

4. (*a*) Cultivated varieties of cotton in Egypt consist of innumerable different strains of cotton.

(*b*) The deterioration which these varieties undergo after a few years in cultivation is due to this heterogeneity through the agency of natural—and unconscious artificial—selection.

5. (*a*) The amount of cross-fertilisation which takes place in the Egyptian cotton fields is not less than five per cent. per annum, nor more than fifteen per cent.

(*b*) The accumulated effect of this annual crossing between the co-existing strains has converted the crop to—or maintained it as—a mass of natural hybrids.

(*c*) This crossing aids in the depreciation of varieties when inferior strains are introduced by seed-mixture.

6. (*a*) Many characters of cotton are intensified on crossing. The causes of this phenomenon are unknown, and the search for them is the most important task in cotton genetics.

(*b*) I cannot resist presenting the speculation that—just as the clovers are hypertrophied and monstrous through the action of nodule bacteria—the improved domestic strains of the higher organisms may be in some respects monstrous through intensification like our cottons.

7. (*a*) On account of climatic and manipulative difficulties, as also the necessity for statistical investigation of the individual, it is impossible to carry out synthetic work in Cotton Genetics unless all facilities for examination of the plants are provided.

(*b*) The Khedivial Agricultural Society has founded the first official Experiment Station devoted to the utilisation of Mendel's Law for economic purposes.

NOTE ON THE ABSORPTION OF ATMOSPHERIC MOISTURE BY CERTAIN NITROGENOUS MANURES.

By GEORGE BROWNLEE, B.Sc.

Albert Agricultural College, Glasnevin, Dublin.

TEN grams each of the manures Sulphate of Ammonia, Nitrate of Soda, Calcium Cyanamide and Nitrate of Lime (Notodden, 1906) were exposed to air in a room the windows of which were kept open during the experiment, and the changes in weight noted for a period of twenty-nine days. At the same time Barometric and Temperature (Wet and Dry Bulb) readings were taken daily.

The percentages of moisture in the manures at the commencement were as follows:—Sulphate of Ammonia 4·52; Nitrate of Soda 2·85; Calcium Cyanamide 0·50; Nitrate of Lime 2·02.

The accompanying chart illustrates the changes in weight and in the atmospheric conditions recorded.

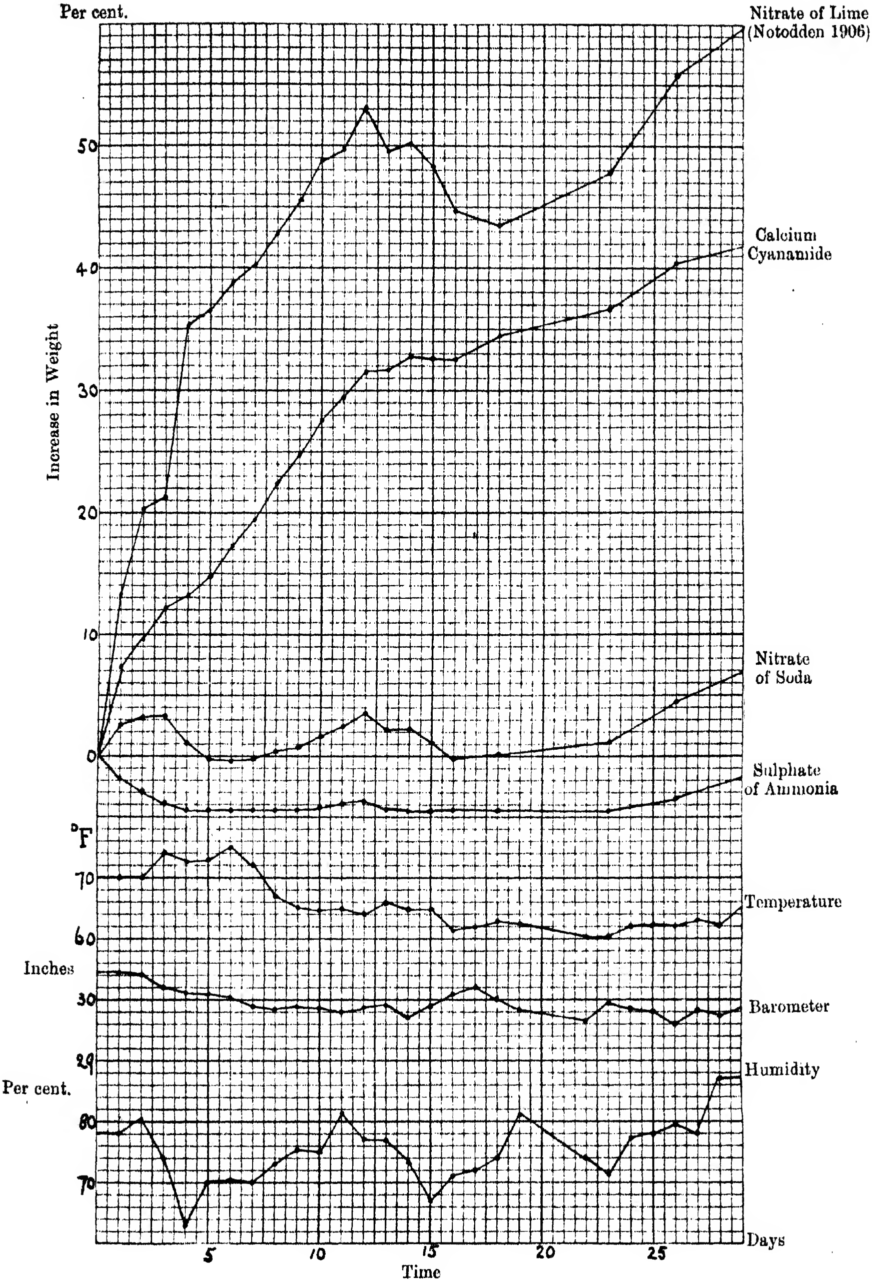
The Sulphate of Ammonia became rather drier during the experiment and was quite powdery at the finish.

The Nitrate of Soda was found at the end to be wettish underneath: apparently its condition varies with the state of the atmosphere. In another experiment on a different sample of the ordinary commercial salt, when the air was very moist for many days in succession, Nitrate of Soda became quite wet. Evidently this manure cannot always be conveniently kept under ordinary conditions.

The Calcium Cyanamide remained unchanged in appearance throughout the experiment. It was, however, partly caked, though quite dry and powdery to the touch. (H. von Feilitzen states[1] that Calcium Cyanamide prepared by the Polzenius process contains a slight excess of Calcium Chloride, which renders the product very hygroscopic, causing it to cake together in hard lumps and to burst the containing sacks.) On opening a bottle containing Calcium Cyanamide there is always a smell of Ammonia showing that this substance would deteriorate if left exposed.

The Nitrate of Lime showed signs of becoming wet after a few hours' exposure and by the third day was quite liquid. It is, therefore, obvious that the Notodden product as made in 1906 must be deemed unsatisfactory from a farmer's point of view.

[1] Abstract in *Jour. Soc. Chem. Indus.*, 26 (1907), No. IX. p. 478.


Per cent.
Nitrate of Lime (Notodden 1906)
Calcium Cyanamide
Nitrate of Soda
Sulphate of Ammonia
Increase in Weight
50
40
30
20
10
0
°F
70
60
Temperature
Inches
30
29
Barometer
Humidity
Per cent.
80
70
Days
5
10
15
20
25
Time

THE COMPOSITION OF GREEN MAIZE AND OF THE SILAGE PRODUCED THEREFROM.

By HAROLD E. ANNETT, B.Sc. (Lond.), S.E.A.C. Dip. *Indian Department of Agriculture* and EDWARD J. RUSSELL, D.Sc. (Lond.) *Rothamsted Experiment Station, late of the South Eastern Agricultural College, Wye.*

Introduction.

The process of making silage is an ancient one[1] and the scientific investigations date back at least to 1873, when Weiske[2], at the Proskau experiment station, showed that there was a loss of carbohydrate, fibre and protein in making silage from sainfoin and other crops. It was, however, by no means general till after the publication in 1875 of Goffart's remarkable success with maize silage at Burtin, in the barren district of Sologne (Loire-et-Cher)[3]. Both Grandeau[4] and Barral[5] analysed Goffart's silage, the former noted the production of volatile and non-volatile acids and labelled them acetic and lactic acids respectively, in which practice he has been followed by most later analysts. Five years later Kellner[6] demonstrated by careful quantitative measurements that the decrease in protein was accompanied by an increase in the amount of "amide" nitrogen. In his experiments about 28 per cent. of the nitrogen was lost, but in a subsequent paper[7] this was traced to volatilisation of ammonia during manipulation of the sample. Kellner considered, and probably correctly, that no nitrogen is lost in the free state from the silo.

Much attention was given to the subject in the wet cycle of years

[1] See *e.g.* Johnston, *Trans. Highland and Agric. Soc.* 1843, new series, 9, 57. A good historical account is given by Jenkins, *Journ. Roy. Agric. Soc.* 1884, 20, 126.

[2] Quoted by Voelcker, *Journ. Roy. Agric. Soc.* 1884, 20.

[3] *Sur la culture et l'ensilage du Maïs-fourrage* (Memoire présenté à la Société centrale d'Agriculture de France, 1875). On p. 8 he says: "Lorsque j'ai acheté le domaine en 1840...huit malheureuses vaches et cent vingt brébis composaient tout le cheptel d'alors et vivaient misérablement...aujourd'hui le même domaine nourrit abondamment soixante-huit bêtes à cornes, six chevaux, et trois cents moutons." He had about 300 acres.

[4] *Ibid.* p. 39.

[5] p. 50 and Part 2, p. 24.

[6] *Land. Versuchs-Stat.* 1880, 26, 447. Mangold leaves were used.

[7] Kellner and Sowano, *Land. Versuchs-Stat.* 1889, 37, 16, also Kellner, *Chem. Zeit.* 1890, 14, 905.

ending in 1883: Fry's letters appeared in the *Agricultural Gazette* and *Mark Lane Express* for 1883 and 1884, and were reprinted in book form in 1885[1]; numerous papers, scientific and practical, were published in 1884 and 1885, and in the latter year a commission sat under Lord Walsingham to collect evidence and report thereon[2]. Experiments were also made at Rothamsted[3]. The scientific papers were mainly analytical and did little more than confirm the earlier results. Weiske and Schulze[4] made maize silage in barrels and observed an increase in the amount of ether extract; they supposed this was due to the conversion of sugar into lactic and butyric acids, both of which are soluble in ether. Analyses were published by Kinch[5], Lloyd[6], Richardson[7], Smetham[8], Voelcker[9], and the late Dr A. Voelcker[10], and served to correct a number of misconceptions that had arisen[11]. As silage never became general in England there have been only few papers published here since 1885.

Meanwhile the subject was being taken up in America. In most parts of the States maize is a far more popular crop than roots, and silage provides the stock with their succulent food in winter. A vast amount of work has been done at the different stations, it is only necessary to mention the investigations of Jordan[12] and Armsby[13] on the digestibility, which proved conclusively the loss of nutritive value; Woll[14], Hills[15], Collier[16] and King[17] on the loss of dry matter; King[17] on

[1] *Sweet Silage*, 1885, Agric. Press Co. London.

[2] The Evidence and Report contain interesting accounts of the methods of making silage, its place in the economy of the farm, and its value here and elsewhere.

[3] *Agric. Gazette*, 1885, also *Rothamsted Memoirs*, Vol. 4, No. 12.

[4] *Journ. für Landwirtschaft*, 1883, 32, Heft. I. Abs. in *Journ. Chem. Soc. Abs.* 1884, p. 1409. Cf. also Palladin, *Ber. der Bot. Gesellschaft*, 1888, 6, 205 and 296.

[5] *Trans. Chem. Soc.* 1884, 45, 122.

[6] *Chem. News*, 1884, 49, 210.

[7] *Trans. Chem. Soc.* 1885, 47, 80.

[8] *Journ. Roy. Agric. Soc.* 1884, 20, 380.

[9] See *Report of Commission*.

[10] *Journ. Roy. Agric. Soc.* 1884, 20, 482, other workers quoted in this paper are Moser and Holdefleiss.

[11] *E.g.* it was often supposed that fibre became digestible during the process and some even considered that sugar was produced. Lawes, in his evidence before the commissioners pointed out the fallacy of supposing that poor coarse grass, weeds, etc. would change into useful, nutritious food in the silo.

[12] *Maine Reports*, 1893 and 1894 (*Expt. Station Record*, 1895, 6, 746 and 1896, 7, 884).

[13] *Pennsylvania Reports*, 1889 (*Expt. Station Record*, 1892, 3, 457).

[14] *Land. Versuchs-Stat.* 1889, 36, 161.

[15] *Vermont Reports*, 1893 (*Expt. Station Record*, 1895, 6, 919).

[16] *New York State Reports*, 1892 (*Expt. Station Record*, 1895, 6, 65).

[17] *Wisconsin Report*, 1894 (*Expt. Station Record*, 1897, 8, 350 and 687: 1898, 9, 393); also *Wisconsin Report*, 1900.

certain physical questions involved; and Babcock and Russell[1] on the bacteriological aspects of the question.

The composition of green maize.

For some years past it has been the practice at the Wye College to grow green maize as a fodder crop and make a certain quantity into silage. The original idea was to see if silage could to any extent take the place of roots, which, on the light chalky soil of the College farm, are often difficult and sometimes expensive to secure. It may at once be stated that green maize was found to be valuable, but silage was only economical in exceptional seasons[2]. The maize was cut during October as opportunity offered, chopped into pieces, and filled into the silo, a cylindrical wooden structure 12 ft. in diameter and 17 ft. high, standing in an extension of the barn. A large sample of the cut pieces (10 or 15 kilos) was drawn, a small subsample taken for analysis, and the bulk weighed into a sack, sewn up and thrown into the silo. This operation was repeated several times during the filling, so that at the end we had several sacks buried at different depths in the silo, each containing weighed quantities of maize of known composition.

The maize is green when cut and far from being ripe. Its composition depends very much on the season; in warm, dry summers there is about 20 per cent. of dry matter, while in cold, wet seasons only about 13 per cent. is found[3], the difference falls almost entirely on the nitrogen-free extract, so that if the nitrogen-free extract and water are added together the sum is very fairly constant. The nitrogen, fibre, and ash show only slight differences from year to year. Most of the nitrogen (about 80 per cent.) is present as true protein, not much amino acid or amide being present at the time of cutting. The nitrogen-free extract contains a certain amount of sugar, which appears to be mainly dextrose, but no starch. The juice is slightly acid to litmus paper and contains gallic acid, but we could find neither lactic, malic, succinic, nor volatile fatty acids. Distillation with hydrochloric acid caused furfurol to be liberated from the pentosans.

[1] *Wisconsin Report*, 1900.

[2] E. J. Russell, *Journ. Board of Agric.* April, 1907, p. 14. Reference is also made to the feeding value of silage, which is not dealt with in the present paper.

[3] The crop varies in the same way, and is much greater in warm than in cold seasons. The difference in the amount of good stuff per acre is therefore very considerable.

TABLE 1.

Average percentage composition of green maize, end of September and early October[1].

	Good seasons		Bad season	Intermediate seasons		Average of all
	1901[2]	1905[3]	1903[4]	1904[5]	1906[6]	
Dry matter	21·90	18·70	13·35	15·00	15·09	16·81
Ether extract	·85	·52	·16	·66	·22	·48
Total nitrogen × 6·25	1·90	2·04	1·81	1·59	1·58	1·78
Nitrogen-free extract	13·60	9·88	6·70	8·06	8·39	9·33
Fibre	4·25	5·30	3·83	3·74	3·93	4·21
Ash..................................	1·30	·98	·79	·95	·97	1·00
Total nitrogen	·304	·328	·289	·253	·252	·285
Protein nitrogen		·267	·210	·212	·166	·214
Non-protein nitrogen (by difference)		·061	·079	·041	·086	·071
°/₀ of total N. present as non-protein		18·8	27·3	16·2	34·1	25·0
Sugar................................		·98	1·5	·81		1·10
Furfurol obtained		2·48	2·01	1·83	1·92	2·06

The crop is never quite even, it is possible on the same day to pick out large, well-advanced plants with hard, woody pith growing alongside of smaller plants, less mature, with soft pith and much more juicy. The difference in composition lies mainly in the water content, the dry matter being much the same in both.

TABLE 2.

Percentage composition of dry matter of (a) large well-advanced and (b) small less mature plants cut the same day.

	Dry matter	Ether extract	Total nitrogen × 6·25	Total nitrogen	Protein nitrogen	Nitrogen free extract	Fibre	Ash	Furfurol	Sugar
(*a*) Mature plants ...	18·95	3·24	10·75	1·72	1·52	55·58	25·01	5·42	11·97	5·74
(*b*) Immature plants.	12·70	4·76	11·00	1·76	1·67	53·29	25·63	5·32	11·74	4·57

[1] For details of the methods employed see the Experimental Part, pp. 389 et seq.
[2] 1 sample. [3] 2 samples. [4] 2 samples. [5] 7 samples. [6] 1 sample.

Composition of Silage.

Some months after filling the silo, the sacks were recovered and their contents weighed and examined. The silage had a brownish-green colour, but otherwise the pieces looked unaltered. It had a pungent smell suggesting butyric acid, and was acid to litmus. Numbers of bacteria were present including *Bac. subtilis* and others, but there was no mould of any sort. The composition is curiously constant, showing far less fluctuation from year to year than does the original maize.

TABLE 3.

Average percentage composition of maize silage.

	1901	1903	1904	1905[1]	1906	Average of all
Dry matter	12·10	12·20	13·79	13·56	13·32	12·99
Ether extract[1]	·16	·10	·83	·56	·29	·39
Total nitrogen[1] × 6·25	1·33	1·35	1·65	1·69	1·30	1·45
Nitrogen-free extract	5·08	5·67	5·15	5·32	5·61	5·38
Fibre	4·66	4·13	5·06	5·06	5·20	4·82
Ash	·98	·95	1·10	·93	·92	·98
Total nitrogen	·213	·216	·265	·269	·208	·234
Protein nitrogen		·121	·156	·181	·089	·137
Non-protein nitrogen (by difference)		·095	·109	·088	·119	·103
% of total N. present as non-protein		43·8	41·1	32·8	57·2	43·72
Nitrogen as NH_3		·003	·006	·014	·006	·007
Nitrogen as amide			·007	·010	·001	·006
Sugar	nil	nil	nil	nil	nil	nil
Furfurol obtained		2·00	1·87	2·26		2·04
Volatile acid (as H_2SO_4)		·17	·07	·03		·09
Non-volatile acid (as H_2SO_4)		·30	·68			·49

When silage is pressed it readily yields quantities of a brownish juice of very complex composition. The amount and nature of the nitrogen compounds in 100 c.c. of a typical sample of this juice were as follows:

Total nitrogen	·161
Nitrogen as NH_3	·030 = 18·6 % of total
Nitrogen as amide	·014 = 8·7 % ,,
Nitrogen as amino acid	·089 = 55·3 % ,,
Nitrogen not accounted for	·028 = 17·4 % ,,

[1] The figures for total and non-protein nitrogen and ammonia are all somewhat too low because we have not been able to avoid loss of ammonia during sampling. We reduced loss as far as possible by starting the several nitrogen determinations in the wet silage immediately the sample was drawn. The ether extract figures are only approximate. See experimental part for details.

A list of the more important compounds present in silage appears in the accompanying paper. It comprises a number of acids containing no nitrogen, which probably have no actual feeding value; among these are formic, acetic, butyric, caproic and hexoic acids, two hydroxyacids, lactic and malic, and also succinic acid. There are also simple nitrogenous compounds, amino acids, basic diamino acids and amides, the feeding value of which is not yet settled, but is certainly less, and probably much less, than that of protein. Finally there are amines which are actually injurious, though whether they normally occur to a sufficient extent to do any harm may be doubted; cases have come to our notice, however, where animals have not thriven on silage and the trouble may very probably be ascribed to these bodies.

It is common to speak of the large amount of "amides" present in silage, but Table 3 shows how very small the quantity really is. Amides occur to a smaller extent than ammonia, and to a still smaller extent than amino-acids. They form only about 5 per cent. of the non-protein nitrogen, and in view of this fact it is highly desirable that the practice of regarding all of the non-protein nitrogen compounds as amides should be discontinued.

The losses going on during ensilage.

On comparing the composition of silage with that of the maize from which it was formed it is possible to discover what has been the loss during the process. An exact quantitative comparison is rendered impossible by the difficulties of sampling and of preventing loss of ammonia, but it is quite clear that a considerable amount of dry matter has disappeared during the process. The loss is not uniform throughout the silo, and no two bags give exactly the same results, but the average of our experiments during 1905 and 1906, set out in Table 4, shows its general distribution.

It will be noticed that the fibre is practically unaltered in amount, and we have confirmed this observation by microscopically examining sections of maize and of the silage afterwards produced. We found that the epidermal cells had undergone no change in the silo beyond a certain amount of shrinkage; even the stomata were unaltered. Similarly the vascular bundles were intact.

The nitrogen-free extract suffers most. Direct tests showed that the sugar disappears almost entirely. The fall in the furfurol indicates a decrease in the amount of cellulose, *i.e.* the less resistant cellulose, which alone falls into this group; this was confirmed by microscopic

examination which showed that many of the cells of the mesophyll were completely disintegrated.

The protein suffers considerably, though not in reality as much as the nitrogen-free extract. Hydrolytic decomposition complicated by bacterial action takes place, and although there is not much, if any, loss of free nitrogen the new nitrogenous compounds are less valuable as food than the protein.

TABLE 4.

Mean losses in the silo 1904 *and* 1905.

	Dry matter	Ether extract	Nitrogen free extract	Fibre	Total nitrogen	Protein nitrogen	Non-protein nitrogen	Ash	Fur-furol
Put in	100	100	100	100	100	100	100	100	100
Brought out..	64	84	45	92	74	45	183	86	68
Loss	36	16	55	8	26	55		14	32
Gain......							83		

The figures show an absolute loss of nitrogen, but we are satisfied that a certain amount of ammonia is given off during sampling, and we have no evidence to show how or to what extent losses of nitrogen occur in the silo. In laboratory experiments on silage made in bottles we only observed losses of nitrogen when air was admitted, and this did not happen in the silo.

The figures given for the loss in ether extract are quite accidental, since the value obtained in any particular bag depends on its position in the silo; the bags in the *top* half showed a considerable loss—32 per cent. on the average—those in the *lower* half showed an average gain of 6 per cent. The ether extract of silage contains a number of soluble acids which obviously wash downwards. Further, since these acids are derived from the nitrogen-free extract or protein of the maize it follows that no comparison is in any case possible.

A similar downwash occurs with the soluble ash constituents, the upper bags losing on an average 17 per cent., and the lower bags gaining 2 per cent., hence the average figure given in Table 4 is of no value. The insoluble ash constituents are of course not liable to this movement; but their amount is so small and variable—depending

partly on the presence of stray soil—that the experimental error becomes too large to give the calculations any value.

The general nature of the losses outlined above is probably the same in all silos, though the actual amount varies: our losses are higher than those observed by American investigators, but there is considerable difference between American and English maize at the time of cutting; ours is much less mature, and contains a lower proportion of nitrogen-free extract.

When a farmer makes silage on a large scale he often wants to know what loss takes place in his silo. The most convincing way of demonstrating this is, of course, to bury weighed bags of material as we have done, but a sufficiently accurate result can be obtained by assuming that the fibre undergoes no change, or only a 5 per cent. loss, and calculating on this basis the amount of each constituent that should be present. This method is much better than another which is sometimes used—viz. to assume that the ash is unchanged in amount and to use it as the basis of calculation; in the first instance, as we have just seen, the soluble ash is liable to wash downwards, and in the second place the percentage of ash is so small that a trifling error in sampling or determination very considerably affects the result. Of course there is in any case the difficulty of getting the average composition of the green material and the silage, which is only got over by taking a number of samples. In our experiments more than one-third of the total dry matter was lost, including more than half the nitrogen-free extract and the protein; in the latter case some of the decomposition products, the amino-acids etc. remained in the silo, so that the actual loss of nitrogen was only 26 per cent. The fibre appeared to undergo no change.

Experimental details.

Analytical methods. The determinations of fat, total nitrogen, fibre, etc., were all made in the ordinary manner. Non-protein nitrogen was estimated by Stutzer's method, the substance is boiled with a mixture of copper hydrate and glycerine which dissolves the non-protein but leaves the protein in the insoluble residue. The method is no doubt open to objection because some proteins may dissolve and some insoluble non-protein bodies, *e.g.* the purin bases, may remain insoluble. But when used for purposes of comparison and not with an idea of getting absolute results we consider that it gives quite valuable information.

To determine the quantities of nitrogen present as ammonia, amide, and amino-acid respectively we adopted the very elegant methods used by Drs Horace Brown and Millar, described in the *Transactions of the Guinness Research Laboratory*, Vol. I. Part 1, 1903. We have found them work very satisfactorily with our subtances.

The furfurol obtained on distillation with hydrochloric acid was converted into its hydrazone and weighed. Krug's method of working was adopted, but we prefer not to attempt expressing our results in terms of any particular pentosan. It is well known that several groups of bodies give furfurol on distillation, including pentosans, celluloses (the so-called "oxy-celluloses"), glycuronic acid, etc., but that certain members of the groups yield instead non-volatile hydroxy-furfurols, and hence cannot be estimated in this way. In view of the fact that the furfurol yielding bodies of green maize have not been carefully examined we prefer to give the experimental figure only, and in the Tables have given the actual weight of furfurol obtained from 100 parts of substance. No doubt the less resistant or "oxy-celluloses" are responsible for much of the furfurol.

Method of procedure. The maize was cut up, roughly dried at about 80° C, ground in a coffee mill and reduced to a fine powder in the Maercker mill. Drying could now be completed, and samples taken for analysis.

A different scheme had to be adopted for silage. As the bags were recovered from the silo they were weighed, brought to the laboratory, and samples taken as speedily as possible for the various nitrogen determinations. But no matter how quickly we worked we could not altogether avoid loss; our figures for total nitrogen and free ammonia are therefore low. Still, the loss is much less than if the samples had been dried before the nitrogen was determined, for the nitrogen found in the dried material is always less than one expects by calculating from the amount in the original wet silage. It will be observed that in three cases the difference is approximately equal to the amount of ammonia present in wet silage, but in the other cases it is much greater.

Samples 1 to 4 were drawn from the 1905 silage, sample 5 was taken in 1906, and sample 6 comes from a silo on another farm.

Another sample of silage was quickly drawn for the determination of the volatile acid. It was distilled in steam, and the distillate titrated with standard alkali. The results are only approximate, the higher acids come over so slowly that the distillate never really becomes

neutral. The non-volatile acid was estimated by titrating the residue in the flask, but we soon gave up this determination because of the difficulty of getting a sharp end reaction.

TABLE 5.

Percentage of nitrogen in the dry matter of silage.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Calculated	1·90	2·10	2·41	2·15	1·58	2·31
Found	1·42	1·61	1·19	1·24	1·45	1·70
Loss during drying	·48	·49	1·22	·91	·13	·61
Ammonia found in wet silage...	·41	·36	·33	·94	·01	·07

A third sample was dried, ground, and used for the estimation of ether extract, fibre, ash, and nitrogen-free extract. The ether extract results are only approximate, samples continue losing weight for days in the extraction apparatus. Some of the acids are only slightly soluble in ether, the colouring matter also dissolves with difficulty.

The bulk of the sample was then used for qualitative examination.

THE CHEMICAL CHANGES TAKING PLACE DURING THE ENSILAGE OF MAIZE.

By EDWARD J. RUSSELL, D.Sc. (Lond.).

Rothamsted Experiment Station.

WHEN the green parts of living plants are cut up and packed in a loosely covered vessel allowing entrance of air, mould soon makes its appearance and decomposition begins: the mass becomes alkaline and is ultimately converted into black humic bodies quite unfit for cattle food. But if air is excluded the change is fundamentally different; no mould developes, the temperature rises, the mass takes on a greenish-brown colour and characteristic odour, it becomes acid and for a long period is suitable for cattle food. The former is a putrefactive change, the latter gives rise to silage.

The general chemical changes known to take place during ensilage are the conversion of sugar and similar bodies into carbon dioxide and water, the production of volatile acetic and butyric acids and of non-volatile lactic acid and the conversion of protein into non-protein[1] material.

Several hypotheses have been put forward to account for the silage changes and to explain why the product keeps so long. According to one—perhaps the commonest—fermentations set up by micro-organisms evolve so much heat that everything is killed and the mass becomes sterilised. Another view is that certain thermophilous organisms bring about the observed changes[2]. Wollny[3] considered that the lactic and

[1] Sometimes called "amides," but it is highly desirable that this term should be dropped. The word "amide" has a definite chemical significance, and many of the nitrogenous non-protein bodies in plants are not "amides" but amino-acids, etc.

[2] *E.g.* Griffiths, *Chem. News*, 1894, 70, 273: see also Lafar, *Tech. Mycology*, p. 262.

[3] *Die Zersetzung der Organischen Stoffe*, 1897.

acetic acids were formed by bacteria but that the rise of temperature was a respiration effect. On the other hand Pasteur's work on anaerobic respiration led Fry[1] to conclude that the changes are not due to bacteria at all but to the cell, and are the result of the altered conditions in which the cell now finds itself. In absence of air oxidation of sugar does not go as far as carbon dioxide and water, but stops at alcohol and acetic acid. This view has been developed by Babcock and H. L. Russell[2] in an important paper published in 1902; they found that silage could be made perfectly well in the presence of ether or chloroform and they therefore conclude that bacterial activity cannot be an essential factor in the process. No acid was produced in this case, however; but acidity appeared when the cells were not killed and the longer the cells lived the more acid was formed.

The first of these hypotheses may be dismissed at once, since silage invariably contains bacteria and is never sterile. Bacteria must therefore obviously play some part, even if only a secondary part, in the process. It is equally clear that the living cell is an active agent. Silage is always made from living plants and the cells live for some time after they are put into the silo; the breaking down processes can still go on though they may be modified by the absence of air, but the building up processes depending on light and air are stopped. Three sets of agents, the living protoplasm, enzymes and bacteria, appear to be involved, and no hypothesis is satisfactory which fails to take account of all three.

The green maize and the silage dealt with in the present paper were produced at the Wye Agricultural College, where a large part of the experimental work was done. The method of making the silage is described in the preceding paper; the details of the separation and the analytical results are in the experimental part of the present one.

The substances produced during ensilage.

On comparing the weights of the various groups of constituents of green maize put into the silo with those of the silage brought out it is found (see preceding paper) that (1) there has been practically no change in the fibre, (2) all of the sugar and some of the less resistant celluloses disappear, (3) carbonic acid is evolved and a number of acids appear, which were not before present, (4) the "protein" nitrogen

[1] *Sweet Silage*, 1885, Agric. Press Co. London.

[2] *Centr. für Bakt.* 1902, 9, 81.

compounds, *i.e.* those forming insoluble compounds with copper hydrate, are reduced to about one-half, (5) the non-protein nitrogen compounds, *i.e.* those forming soluble copper compounds, practically double in amount.

The changes undergone by the nitrogenous compounds are well seen in the juices pressed out from maize and from silage respectively; the latter contains more substances reacting with nitrous acid and with phosphotungstic acid than the former.

	Weights in 100 c.c. of juice		Relative weights, total N. = 100	
	Fresh maize juice, grams	Silage juice, grams	Fresh maize juice	Silage juice
Total Nitrogen	·0677	·161	100	100
N. liberated by HNO_2[1]	·0183	·089	27	55
N. precipitated by phosphotungstic acid	·0108	·060	16	37

The relative amounts of the different groups of nitrogen compounds in silage vary somewhat, but the juice referred to in the preceding table contained in 100 c.c.:

	Grams	Percentage, total = 100
Nitrogen as ammonia	·030	18·6
Nitrogen as amide	·014	8·7
Nitrogen as amino-acid	·075	46·5
Nitrogen not accounted for	·042	26·2
Total	·161	100

These results indicate the general nature of the changes; we can, however, get more definite information by ascertaining what compounds are actually present. The following have been isolated by the author from silage:

Alcohols	*Fatty acids*	*Hydroxy-acids*	*Dibasic acids*
Ethyl alcohol	Formic acid	Lactic acid	Carbonic acid
	Acetic acid	Malic acid	Succinic acid
	n-Butyric acid		
	Isopropylacetic acid		
	Hexoic acid (*either* *n*-caproic *or* isobutylacetic acid)		

Nitrogen compounds

Mono-amino acids	*Diamino acids*	*Basic compounds*
A mixture which could not be separated. Reactions indicate	Histidine	Ammonia
	Lysin	Pentamethylene diamine
	Ornithin	Betain (trace only)
glycocol		Adenin
alanine and others		Another purin base not identified

[1] In the apparatus designed by Drs Brown and Millar (*Trans. Guinness Research Lab.* 1903, 1, 30).

In addition there was some gummy matter, some lecithin, a fragrant oil, a little humus, besides the usual celluloses, protein, oil, etc.

The following were sought for, but could not be found: aldehyde, higher alcohols, glycerin, arginin.

Many of these are altogether absent from maize, *e.g.* the volatile acids, lactic acid, etc.; others are found, *e.g.* the amino acids, but to a much less extent.

This list does not include all the constituents, for indications of other bodies were obtained; but it will be found sufficient to give a fairly accurate idea of what goes on in the silo.

The agents producing the changes.

It has already been stated that there are three possible agents likely to be involved in bringing about changes in the silo, viz., living protoplasm carrying on its vital functions particularly respiration, enzymes which, though originating from protoplasm, can nevertheless act independently of it, and micro-organisms. The relative parts played by these three cannot be ascertained by direct experiment because of the impossibility of sterilising pieces of living maize, hence the effect of the cell alone, apart from organisms, cannot be investigated. The method adopted in the first series of experiments was to pack into bottles (which make very good miniature silos) pieces of (A) living maize, to get the total effect of all three agents, (B) maize killed and sterilised with toluene, which still allows enzymes to act, (C) maize killed by heating to 98° C., when enzymes and living organisms are destroyed but not spores; certain organisms may therefore develope, (D) living maize inoculated with silage juice containing large numbers of the organisms actually occurring in the silo. In all these cases the bottles were closed to prevent entrance of air but provision was made for the escape of gases. Further, in order to observe the effect of admitting air some of the bottles containing living maize were simply plugged with cotton-wool (E). The bottles were then kept for five months at 20° C. in an incubator. At the end of the period the bottles were opened and the contents examined. The results may be summarised as follows:

Air excluded				Air admitted
A	B	C	D	E
Protoplasm, enzymes and casual organisms all acting. (Living maize)	Enzymes only acting. (Maize and toluene)	Protoplasm and enzymes killed: spore-forming organisms present. (Maize heated to 98° C.)	Protoplasm, enzymes and silage organisms present. (Maize and silage juice)	(Living maize)
Silage formed	No obvious change	No obvious change	Silage formed	Putrefaction
No mould	No mould	Little mould	No mould	Much mould
Some dry matter lost (25 %)	No loss	Little loss (12 %)	Some loss (25 %)	Much loss (60 %)
Mass becomes acid	No change	No change	Mass becomes acid	Mass becomes alkaline
Volatile acids (acetic and butyric) formed	None formed	None formed	Acetic and butyric acids produced	None formed
Protein changes to non-protein	Protein changes to non-protein	No change	Protein changes to non-protein	Non-protein changes to protein
No loss of nitrogen	No loss of nitrogen	No loss of nitrogen	No loss of nitrogen	Nitrogen lost

It will be observed that the characteristic silage changes, the production of the silage odour and colour, of volatile acids and the conversion of protein to non-protein, occur only in A and D where living cells are kept out of contact with air. If cells and organisms are killed, but enzymes are allowed to act, only one of the changes takes place—the conversion of protein to non-protein. If spore-forming organisms alone survive, as in C, none of the silage changes occur.

The volatile acids. Since these are formed only in A and D and not at all in B or C their formation must be attributed either to the living protoplasm of the maize cell or to casual organisms; their invariable and rapid production in A (this experiment was repeated a number of times) appears strongly to indicate that they are formed by the living cell. Further, their non-occurrence in E shows that they arise only when the living cell is deprived of oxygen. They may therefore be regarded as products of anaerobic respiration. But although the living cell is probably the primary agent, the organisms also play a part in either producing or modifying them; the acids in D, where organisms were introduced, were somewhat different in character from those in A.

Complete separations were not attempted, but the silver salts of the mixtures contained the following percentages of silver:

	Mixture in A (living maize only)	Mixture in D (living maize + silage organisms)
Bottle 1	56·56	60·21
Bottle 2	57·52	

Silver acetate contains 64·65 per cent. and silver butyrate 55·40 per cent.

It appears that more of the lower homologues are present in D than in A.

The mixture found in the silo consists mainly of butyric and acetic acids, but there occur also formic acid and some of the higher acids. Formic acid and the higher acids are known to be the products of the bacterial decomposition of cellulose, and as cellulose disappears during ensilage one can safely attribute part of the volatile acid to organisms. These decompositions are, however, quite secondary.

The conversion of protein to non-protein. Unlike the formation of volatile acids the decomposition of protein does not depend directly on the living cell, for we find it going on in B, where the cells had been killed by toluene. It does not, however, occur in C, where the enzymes had been decomposed, and this change is therefore to be attributed to enzymes which can act not only during the life, but also after the death of the cell. The amount of change observed was:

	A (maize only)	B (maize and toluene)
Protein N. put in	100	100
Protein N. brought out	75	89
Protein N. converted into non-protein	25	11

The quantity of protein decomposed is less when the cells are suddenly killed by toluene than when they continue living for some time, but this is quite consistent with the general facts of enzyme action. The killed cells contain only the amount of enzyme present at the moment of death, action must therefore stop as soon as this has acted on the protein in its immediate vicinity; moreover the toluene may retard its action. In A, on the other hand, fresh quantities of enzyme can always be made so long as the cell lives, and the presence of a little acid is known to be favourable to protein hydrolysis.

Further evidence of the presence of enzymes capable of decomposing protein was obtained by studying the changes in sterilised maize juice. Some green maize was cut in October at the time the silo was being filled, it was pressed, and the juice, to which 2 per cent. of toluene was added, was kept at 25° C.; after some time it was found to have undergone a considerable amount of hydrolysis, for there was a great increase in the compounds decomposed by nitrous acid and in those precipitated by phosphotungstic acid. Action has been more vigorous than in the last experiment, probably because diffusion has gone on better.

	In fresh maize juice	After being kept at 25°
Total N.	100	100
N. liberated by nitrous acid	27	55
N. in phosphotungstic acid precipitate	16	33

As 2 per cent. of toluene was found to keep the juice sterile (for no growth was obtained on inoculating a little into bouillon) this hydrolysis can only be attributed to enzymes. It is therefore evident that the maize put into the silo contained enzymes capable of hydrolysing the protein of the cell even after the cell is dead.

The change that has gone on in the silo clearly indicates that such a hydrolysis has actually taken place and is to be regarded as the primary cause of the decomposition of protein during ensilage. In the first place the average general grouping of the nitrogen compounds in silage (see p. 394) is much the same as that in the hydrolysed maize juice just referred to; nitrous acid liberated 55[1] per cent. of the total nitrogen in each case, and phosphotungstic acid precipitated 37 and 33 per cent. respectively. In the second place typical products of proteolysis occur in the silo. When protein is hydrolysed by tryptic enzymes the products include mono- and di-amino acids (lysin, histidin, etc.). When nucleo-proteins are hydrolysed the purin bases are obtained in addition. All these compounds are found in silage.

It would not, however, be correct to ascribe the whole change to enzymes. The organisms present are not without action on the nitrogen compounds, and certain bodies, *e.g.* amines characteristic of bacterial action, are found in the silo. But the bacterial changes appear to be secondary and not an essential part of the process.

[1] 55 per cent. was the mean amount obtained from silage juice; the actual amount, however, varied considerably in different samples from 38 per cent. to about 65 per cent.

The influence of free oxygen on the process. The effect of allowing free oxygen to have access to the maize in E (p. 396) was to alter the product completely. There was a great development of Penicillium, which did not appear when air was excluded. The mass became black and had a musty smell, it was alkaline and appeared to be free from acetates and butyrates; there had been an increase in the amount of protein, indicating that some had been formed from the simpler non-protein material, presumably by the mould. The general changes were:

	Dry matter	Protein nitrogen	Non-protein nitrogen	Total nitrogen
Put in	17·95	·197	·104	·301
Brought out	7·00	·238	·041	·279
Loss	10·95	– ·041	·063	·022
Percentage loss	61·0		61·5	7·3
" gain		20·8		

It will be remembered that in A and B where air was excluded, the protein had decreased 25 and 11 per cent. respectively, whilst here it has increased 21 per cent.

The change in the non-protein substances was also shown directly by determining the amount of nitrogen liberated by nitrous acid. In the case of good silage juice more than 40 per cent. of the total nitrogen is set free by this reagent; while only seven per cent. is liberated from juice expressed from the black, alkaline mouldy layers found at the top of the silo, stretching down as far as the air can enter. Again, some fresh silage juice, which gave up 38 per cent. of its nitrogen on treatment with nitrous acid was allowed to become mouldy; after three months only 18 per cent. was set free.

The disappearance of non-protein, and the formation of protein, indicates that the moulds and other organisms have utilised the simpler substances as food, and built them up into complex cell constituents. A similar change is known to take place when farmyard manure is stored.

The chemical effects following on the admission of air can be ascribed to the course of respiration in the cell, which now remains normal, so that butyric and acetic acids are no longer formed in quantity, and to the development of mould, which appears in the black decomposed layers at the top of the silo as far as oxygen can get in but not lower

down. Indeed the absence of mould is characteristic of good silage, though it is very difficult to explain. Penicillium is reckoned among the hardiest forms of life, it seems able to grow almost anywhere and to tolerate bodies that would be fatal to most other organisms, yet it cannot grow in the silo, and at a certain distance down it stops absolutely short. On the dividing line where the black mouldy layer ends and the good silage begins the author has often found pieces of maize one inch long, one end of which was strongly acid and free from mould, while the other was alkaline and had a growth of Penicillium. It would appear that there is some actual inhibiting agent produced in the silo when oxygen is absent and not formed when oxygen is present, though no doubt the absence of free oxygen in the silo is also a cause why mould does not develope.

The course of the process in the silo.

Putting together our results it is possible to sketch out fairly completely what happens in the silo. When the cells are put in they are alive and their vital functions continue. Respiration goes on and sugar, etc., is used up, but in absence of air oxidation is not complete, and intermediate bodies, alcohol, acetic acid, butyric and other acids are formed in addition to carbon dioxide and water. The tryptic enzymes of the cell act on the protein forming the usual hydrolytic products, amino acids, diamino acids, etc.: from the nucleo-proteins purin bases are produced in addition. The heat developed during these processes cannot be dissipated, as it usually is in the living plant by the evaporation of water, because water vapour cannot escape from the silo; instead it raises the temperature of the mass. Respiration is accelerated by the increased temperature, but, as no more material is being elaborated and only decomposition is taking place the process soon comes to an end; the cell then dies for want of more substance to break down, it loses its turgidity, and becomes flaccid, causing the mass to settle down. The temperature also steadily falls[1]. The decomposition of the protein, which had also been accelerated by the rise in temperature, can continue even after the cell is dead, because the enzymes when once formed are not dependent on the life of the cell, in other words, it is an autolytic decomposition. Some of the products formed in the above changes inhibit the development of mould, and the general conditions obtaining

[1] The maximum of 33° to 37° C. observed in the Wye silo was always reached in about 5 days; there was then a slow but regular fall.

in the silo are unfavourable to putrefaction. The mass therefore remains good for food for a long time.

It is, however, not sterile. Certain bacteria are always present and attack the softer celluloses (but not to any extent the resistant "fibre") producing the humus, some of the fatty acids, and probably the succinic acid present in silage. They also carry several stages further the decomposition of certain of the nitrogen compounds, and produce those bodies which cannot be supposed to arise from tryptic hydrolysis of protein. Thus pentamethylene diamine is a well-known decomposition product of lysin; ornithin is known to arise from arginin, and the absence of the latter body may be due to its decomposition into ornithin and urea, which would further change into ammonium carbonate.

In the following scheme are set out the changes as the author supposes them to take place. The lactic and malic acids might have arisen from the amino acids or from the carbohydrate material, the present experiments do not enable one to decide this point.

On the above hypothesis the changes essential to the production of silage—the disappearance of sugar, the formation of volatile fatty acids and the hydrolysis of protein—are all considered to be the result of the activity of the living protoplasm or of the enzymes of the maize cell. The other changes due to the bacteria invariably present are considered to be of purely secondary importance and merely have the effect of complicating the product without greatly altering its nature or even masking the primary changes.

EXPERIMENTAL PART.

ISOLATION AND IDENTIFICATION OF THE COMPOUNDS PRESENT IN SILAGE.

1. *Non-nitrogenous compounds.*

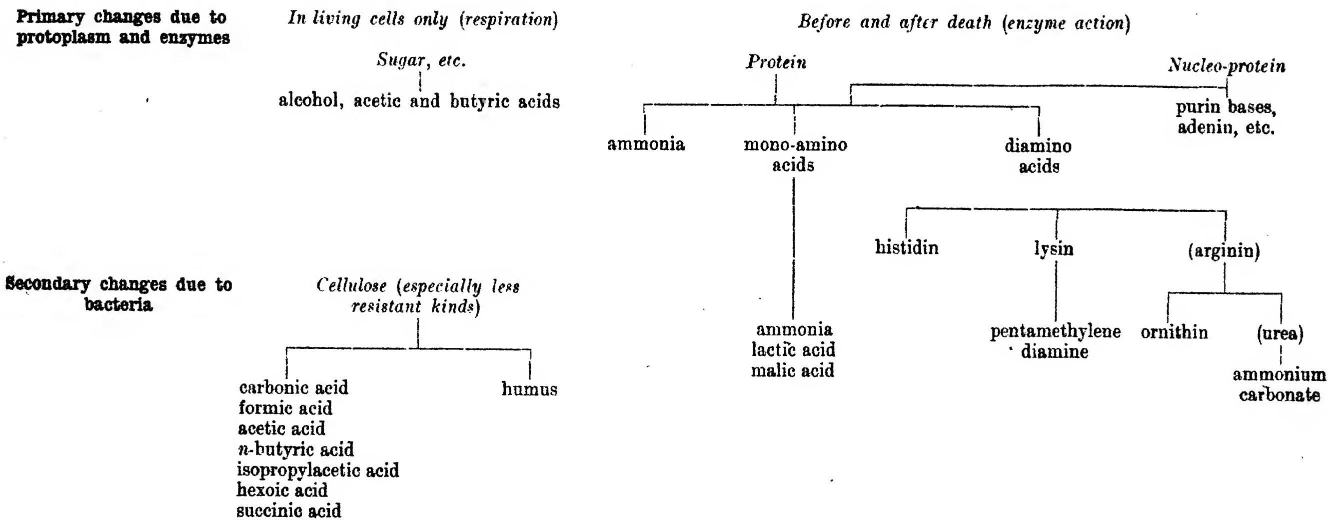
Ten kilos of silage were distilled in steam and ten litres of distillate collected. Towards the end of the process ·5 per cent. of sulphuric acid was added to set free any combined acids.

The distillate (A) contained alcohol, volatile acids, etc.

The residue in the flask (B) contained non-volatile acids.

Examination of distillate A. Excess of silver oxide was added to

SCHEME SHOWING CHANGES DURING ENSILAGE.



The substances in brackets are supposed to have been formed as intermediate products, though they could not be detected in the silage.

combine with the acids and the whole was distilled. The distillate (C) contained non-acid bodies, the residue (D) was composed of silver salts of the volatile acids.

The non-acid bodies in C. This had the characteristic maize smell. A portion tested with Schiff's reagent gave no colour. Aldehyde is therefore absent.

Another portion was treated with potassium permanganate and sulphuric acid. Aldehyde was formed, it was distilled off and gave an intense Schiff reaction. A **primary alcohol** is therefore present.

On treatment with iodine and sodium hydrate a precipitate of iodoform was obtained. The crystals were seen under the microscope to form the star-shaped clusters characteristic of **ethyl alcohol**[1].

Six litres of the distillate were evaporated on the water bath, there was a residue, but it contained neither glycol[2], glycerine[3], nor fusel oil[4]. It gave no aldehyde on oxidation, but yielded an acid which, however, was too small in amount for investigation; it was not oxalic acid.

The rest of the distillate was extracted with ether previously washed and distilled over sodium to remove alcohol. After distilling off the ether a small pleasant smelling residue was left which was apparently an ester. There was no evidence of fusel oil.

The **absence of glycerine** and **higher alcohols**, except in traces, is therefore probable.

The volatile acids in D. The silver salts were extracted with hot water and boiled for a few minutes. Reduction took place, indicating the presence of **formic acid**. This was confirmed by treating a new lot of distillate with mercuric chloride and digesting at 90° C. for some time, when mercurous chloride was precipitated.

The solution of silver salts was filtered, concentrated, and the crystals taken out in four fractions. Each fraction was similarly broken up into two others which were purified by recrystallisation, the separate fractions being united when they were evidently identical.

The first crop of crystals was small, and was found to be a silver **hexoate**,

Ag found = 48·74 per cent., $C_6H_{11}O_2Ag$ requires 48·43 per cent.

[1] A large number of bodies react with iodine and sodium hydrate to form iodoform, but under the high power of the microscope this is seen to form hexagonal plates excepting when ethyl alcohol is present, in which case the crystals are star-shaped.

[2] Glycol gives oxalic acid on oxidation.

[3] Dunstan's test was used, *Pharm. Journal* [3], vol. 14, p. 41.

[4] The higher primary alcohols give aldehyde on oxidation. Fusel oil is insoluble in ether and this substance readily dissolved.

The intermediate crops yielded a little **valerate**.

Ag found = 51·9 per cent., $C_5H_9O_2$ Ag requires 51·68 per cent., and a large amount of a **butyrate**,

Ag found = 55·83 per cent., $C_4H_7O_2$ Ag requires 55·40 per cent.

The last crops yielded silver **acetate**,

Ag found = 64·40 per cent., $C_2H_3O_2$ Ag requires 64·65 per cent., while the mother liquors contained a small amount of what appeared to be silver lactate.

It is not possible, however, to discriminate between the isomers by means of the silver salts. In order to do this a fresh lot of the acids was prepared from silage and converted into sodium salts; these were dried, well ground and suspended in alcohol, a current of hydrochloric acid gas was then passed through the mixture. The esters thus obtained were fractionated, and the separate fractions on redistillation yielded small amounts of liquids with fairly constant boiling points. The liquids were identified by molecular weight determinations in a Victor Meyer's apparatus.

The hexoic ester was found to be either *n*-**caproic** or **isobutyl acetic** ester.

Mol. wt. found = 139, theor. 144. B. pt. of fraction = 160°—170°. B. pt. of isobutyl acetic ester = 161°, of *n*-caproic ester = 167°.

The small amount of hexoic ester was insufficient for further examination and it is not possible to say which of the two isomers is present.

The valeric ester was found to be either methyl ethyl acetic ester or isopropyl acetic ester.

Mol. wt. found = 126, theor. 130. B. pt. of fraction = 132°—135°. B. pt. of methyl ethyl acetic ester = 133·5°, of isopropyl acetic ester = 135°.

The ester was dissolved in chloroform and examined in a polariscope. It was found to be optically inactive and is therefore **isopropyl acetic ester**.

The butyric ester was found to be *n*-**butyric** ester.

B. pt. found = 119°—121°. B. pt. of *n*-butyric ester = 120·9°.

Small amounts of a high boiling substance were found, and were probably ethyl lactate.

Mol. wt. found = 119. Mol. wt. of ethyl lactate = 118. B. pt. found = 150°—160°. B. pt. of ethyl lactate = 154·5°.

The non-volatile acids in B. The liquid was acidified with sulphuric acid, decanted from the silage, and extracted repeatedly with ether.

The ether was then distilled off, water was added and calcium carbonate in excess, and the calcium salts obtained were evaporated to dryness on the water bath. They were then extracted with 90 per cent. alcohol, and the residue was treated with water. Part remained fairly insoluble.

(*a*) *The salt soluble in alcohol.* This had a brown colour and could not be crystallised, it was accordingly dissolved in alcohol and precipitated by ether. Repetition of the process two or three times gave a white salt crystallising in the tufts of needles characteristic of calcium **lactate.** This was dried at 100° and analysed,

CaO found = 25·50, calculated for $Ca(C_3H_5O_3)_2$ 25·69.

(*b*) *The salt readily soluble in water.* This also was brown; it was dissolved in a small amount of water and precipitated by alcohol, the process being repeated several times. The pure salt was found to be calcium **malate.** It was dried at 100° and analysed,

CaO found = 29·30, calculated for $CaC_4H_4O_5 . H_2O$ 29·47.

As malic acid had not before been found in silage we confirmed its presence by preparing and analysing the silver salt, this being made from the calcium salt,

Ag found = 61·63, calculated for $Ag_2C_4H_4O_5$ 62·07.

Further confirmation was obtained by preparing the lead salt, and by isolating the acid and converting it into malic acid.

(*c*) *The salt less soluble in water.* This differed from the previous salts in giving a buff coloured precipitate with ferric chloride. Analysis of the salt dried at 100° showed it to be calcium **succinate.**

CaO found = 32·32, calculated for $CaC_4H_4O_4 . H_2O$ 32·18.

(*d*) *The residue insoluble in water.* This contained neither oxalic nor tartaric acids, and appeared to be only calcium carbonate with a little sulphate.

2. *The nitrogenous compounds.*

As it was only proposed to seek for soluble nitrogen compounds the author used the juice squeezed out from fresh silage, and adopting a slight modification of Schulze's method as described in *Landw. Versuchs-Stat.* 1904, Vol. 59, p. 344.

1. The juice was first treated with basic lead acetate[1] to precipitate

[1] Prepared by boiling a 30 % solution of normal acetate with excess of finely ground litharge, allowing to cool, and filtering.

the finely divided matter floating in the liquid and also the gummy bodies vaguely known as extractives. The precipitate (A) was filtered.

2. The filtrate from A was treated with hydrogen sulphide to remove lead, then concentrated and treated with a 10 per cent. solution of phosphotungstic acid in five per cent. sulphuric acid. The precipitate (B) may contain the basic diamino acids, arginin, histidin, lysin, and ammonia, amines, betain and cholin.

3. The filtrate was treated with mercuric nitrate and caustic soda was added till the solution was faintly alkaline. The precipitate (C) may contain mono-amino acids, ureids (*e.g.* allantoin), etc.

The lead acetate precipitate (A) was found to contain only ·85 per cent. of nitrogen, evidently as protein. It gave the xanthoproteic test, but no tryptophane or murexide reactions, and was not further examined.

Examination of the mercury precipitate (C). This was suspended in water and decomposed with sulphuretted hydrogen, the solution was then concentrated and left to crystallise. No crystals could, however, be obtained, but only a syrup, with a strong glue-like smell. It readily yielded brightly coloured copper salts with copper hydroxide, but all attempts to resolve it into its constituents failed. No tyrosin reaction with Millon's solution was obtained. Ferric chloride gave a red colour which indicates the presence of **glycocol** (amido-acetic acid) and nitrous acid produced a mixture from which lactic acid was isolated, indicating that **alanine** (and amido-propionic acid) is present. More definite proof could not, however, be obtained.

Examination of the phosphotungstic acid precipitate. For the preliminary tests Kossel's method was used[1]. The precipitate was suspended in water in a mortar, and ground with successive quantities of barium hydrate till the liquid had an alkaline reaction. The barium combines with the phosphotungstic acid, and liberates the bases which pass into solution. After filtering, carbon dioxide is passed through the liquid till the dissolved baryta is all precipitated, it is filtered and the filtrate saturated with carbon dioxide. Mercuric chloride is then added till the mixture becomes acid. A precipitate was produced indicating the probable presence of histidine (D).

This was filtered, the mercury in the filtrate was removed by sulphuretted hydrogen, and after again filtering, the chlorine in the filtrate was removed by silver sulphate. The filtrate, which now

[1] *Zeitschrift für Physiolog. Chemie* (Hoppe-Seyler), 1896, 22, 177 and 1898, 26, 586.

contains the bases as sulphates, was warmed, and treated with silver sulphate till a small test portion gave a brown precipitate with baryta. Baryta was then added to the bulk of the liquid till precipitation was complete. The precipitate would contain arginin, if present (E).

The filtrate is treated with CO_2 to remove barium, and with HCl to remove silver. An alcoholic solution of picric acid was then added taking care to avoid excess, this gave a beautifully crystalline precipitate indicating the presence of lysin (F).

The filtrate was treated with hydrochloric acid and extracted with ether to remove picric acid. Phosphotungstic acid was added and produced a copious precipitate, showing that other bases were present. These were liberated, as before, by treatment with baryta and subsequent treatment with carbonic acid. They were then converted into their hydrochlorides and concentrated.

Gold chloride was now added. A precipitate was produced indicating the presence of other bases.

The mercuric chloride precipitate D was decomposed by suspending in water and treating with a current of sulphuretted hydrogen. On evaporation a few crystals were obtained which gave a magnificent cherry red colour with an alkaline solution of diazobenzene sulphanilic acid characteristic of imido-azol compounds having the hydrogen in the (7) position not replaced. Histidin shows this reaction.

The silver precipitate was decomposed by sulphuretted hydrogen and the filtrate concentrated. The solution gave no precipitate with phosphotungstic acid, and it is therefore probable that **arginin** is **absent.**

The picrate was decomposed with hydrochloric acid and extracted with ether to remove picric acid. On evaporation of the aqueous layer crystals of a hydrochloride soluble in methyl alcohol were obtained. This appeared to be **lysin** hydrochloride.

The original solution of the bases was also found to give the reactions of the purin bases. Ammoniacal solution of silver nitrate and a mixture of sodium bisulphite and copper sulphate both gave precipitates; a yellow residue was also obtained on evaporation with nitric acid, which turned purple when treated with caustic soda.

The method adopted for the isolation of the bases was as follows:

The phosphotungstic acid precipitate was, as before, ground up with baryta, excess of which was subsequently removed. The solution containing the free bases was just acidified with nitric acid and concentrated; it was then made distinctly acid with nitric acid and treated with silver nitrate. A white precipitate was formed (E).

To the filtrate ammonia was added till the solution was barely alkaline. A white precipitate (F) was obtained.

E gave the reactions of the purin bases, but it contained 64·4 per cent. of silver, which does not correspond with any of their ordinary silver salts. The amount obtained was too small to allow of further examination.

F proved to be silver histidin salt obtained by Hedin, $C_6H_7Ag_2N_3O_2$, H_2O,

Silver found = 55·79, theor. 55·82.

Histidin is therefore present.

The filtrate from F was now treated with phosphotungstic acid and the bases once more obtained in the free state. They were strongly alkaline and had an exceedingly disagreeable odour characteristic of the amines. The lysin was precipitated as picrate, the picric acid was removed from the solution and the bases were converted into hydrochlorides and evaporated to dryness; the residue was extracted with absolute alcohol.

Examination of the hydrochlorides soluble in absolute alcohol. This might contain cholin, cadaverin, putrescin, etc. Addition of alcoholic platinum chloride produced a precipitate which on purification and ignition yielded 37·72 per cent. of platinum. The precipitate therefore appears to be the platinum salt of **pentamethylene diamine** (cadaverin) which contains 38·06 per cent. of platinum. No other platinum salt appeared to be mixed with it, the separate fractions all containing practically the same amount of platinum.

Examination of the hydrochlorides insoluble in absolute alcohol. This was dissolved in a little water and tested with gold chloride. A precipitate was obtained, which however was quite different in character from the ordinary betain compound. The hydrochloride was found to give a precipitate with mercuric nitrate and to form a copper salt, it was therefore a diamino acid, presumably **αδ diaminovaleric acid** (ornithin) since it also gave a precipitate with mercuric chloride. The gold salt was prepared and analysed,

Au found = 38·88, calculated for $C_5H_{12}N_2O_2 . 2HCl . AuCl_3$ 38·76.

The filtrate still contained bases, but their nature has not been ascertained. A reducing body is present, since metallic gold was deposited from the solution on standing. Traces of betain were found on one occasion to be present.

The purin bases. Owing to the small quantity present in the juice it was necessary to examine the solid part of the silage. This was extracted

with water to remove easily soluble bodies and then with dilute nitric acid. The extract gave the reactions characteristic of purin bases—precipitates with ammoniacal silver nitrate, with copper sulphate and sodium bisulphate, and the usual colour reactions on evaporating with nitric acid and moistening with caustic soda, but the amount obtained was too small for complete examination. On treating with mercuric nitrate a precipitate was obtained from which a small quantity of base was isolated which gave the silver and copper precipitates, gave a red colour with diazobenzene sulphanilic acid, sublimed on heating and gave a crystalline gold chloride. These are also the reactions shown by **6-amino-purin (adenin)** which therefore appears to be present.

Au found = 37·0, calculated for $C_5H_5N_5 . 2HCl . AuCl_3 . H_2O$ 37·2.

Since, however, adenin does not give the xanthin colour test it is clear that another purin base is also present.

After the nitric acid was removed from the silage ammonia was added and a dark-coloured, fluorescent solution was obtained. This also gave the reactions characteristic of the purin bases, but it also contained a certain amount of humus, which proved so troublesome that further examination was not made.

The following substances were tested for, but not found :—allantoin, kreatin, kreatinin.

Conclusions.

1. The main groups of compounds found in maize silage are fatty acids, hydroxy- acids, amino acids, basic diamino acids, purin bases, and other bases, besides the ordinary constituents of the plant cell, the celluloses, protein, etc. The non-nitrogenous acids are not found in maize at the time of cutting, and the nitrogenous acids, though they are found, occur to a smaller extent than in silage.

2. The characteristic silage changes are the disappearance of sugar, of some less resistant celluloses, and of part of the protein, and the formation of the bodies enumerated above.

3. Three agents appear to be involved in making silage, the living maize cell, the enzymes, and micro-organisms. It is considered that the two former bring about the primary and essential changes, the latter only secondary and non-essential changes.

4. The formation of acetic and butyric acids appears to be a respiration effect, and comes about when the living cell is deprived of oxygen. Sugar disappears during the process.

The decomposition of the protein and nucleo-protein is effected by enzymes present at the time of cutting the maize, which can go on acting in the silo even after the cell is dead. Characteristic products of protein-hydrolysis were identified in the silage.

These are regarded as the primary and essential changes.

5. Bacteria are, however, always present, and attack the less resistant celluloses, the products of protein-hydrolysis, and no doubt other substances as well, but not the resistant fibre. Typical products of bacterial activity were found—formic acid, higher fatty acids, humus and amines.

6. The growth of mould is inhibited except at the surface layer where air gets in. Here the changes are fundamentally different; there is no development of acetic or butyric acids, the mass is alkaline, non-protein material already existing in the maize is converted into protein, and there is also a loss of nitrogen.

STUDIES ON GERMINATION AND PLANT-GROWTH.

By SPENCER UMFREVILLE PICKERING, M.A., F.R.S.

EXPERIMENTS which have been in progress at the Woburn Experimental Fruit Farm on the effect of grass on trees, have led to the conclusion that this effect cannot be attributed to any such simple causes as root-competition, increased evaporation, or differences in temperature or aëration of the soil, but must be due to some poisoning action exerted by the grass. Whether, however, such action is a direct result of the growth of the grass, or an indirect one, operating through the medium of soil-bacteria, there is, at present, no evidence to prove.

Behaviour of trees in heated soil.

As an initial experiment on this aspect of the case, 26 two-year old apple trees were planted in pots under various conditions, including the use of heated soil. The trees were planted in February, and were kept out in the open after planting; three of them were in soil from Ridgmont (see p. 427) which had been heated for two hours at 82°, and six in soil heated to about 200°, the soil in three out of these six having been previously powdered and sifted. The sterilisation of the soil at 82° would, of course, be only partial, and in all cases there would be opportunity for re-inoculation to occur. The pots were kept constant in weight by additions of water every other day, and no artificial nourishment was given.

One of the nine trees in heated soil almost died, but the other eight soon became conspicuous for the extra growth which they were making, as well as for the darker colour of their leaves, and these eventually remained on the trees two or three weeks after the normal date of falling. Below are given the length of new wood formed, the total weight of the leaves, the percentage of nitrogen in the dried leaves, and the total dry matter and nitrogen in the leaf-crop: the actual quantities, where given, are in

grams; the relative values are obtained by comparison with those given by the trees in unheated soil. For the nitrogen determinations I am indebted to Dr E. J. Russell, who had them made in the Lawes' Laboratory by the kind permission of Mr A. D. Hall.

TABLE I. *Apple trees (King of the Pippins) grown in heated and unheated soil.*

Soil	Length of new wood. Relative		Weight of leaf-crop. Relative		p.c. nitrogen in leaves. Actual; Rel.	Dry matter in leaf-crop. Actual; Rel.	Nitrogen in leaf-crop. Actual; Rel.
Unheated	100 107 92	100	90 100 110	100	1·735 = 100	21·6 = 100	·375 = 100
Heated to 82°......	88 190	139	109 172	141	1·826 = 105	31·1 = 144	·576 = 151
Heated to 200° ...	190 102 176	163	180 126 137	148	2·036 = 117	32·8 = 152	·667 = 178
Ground and heated to 200°	144 90 151	128	190 108 112	137	2·446 = 141	26·0 = 120	·635 = 170

The trees in soil which had been heated show a much greater development both of wood and leaf than those in unheated soil, also, the leaves in the former case are the richer in nitrogen, so that the total nitrogen assimilated shows an excess of as much as 50 to 80 per cent. All the properties examined show that the heating at 200° has been more beneficial than that at 82°, but, where the soil was ground as well as heated, the indications are less uniform, the leaves being richer in nitrogen than in any other case, but the dry matter in them, and also the length of wood formed, being comparatively low. No doubt the beneficial effect of heating has been facilitated by the grinding, but the fine state of division of the soil has interfered with the proper functioning of the roots through lack of free aëration. There is much less uniformity, it may be observed, between the duplicates in heated soil than between those in the unheated soil: a similar lack of uniformity in the behaviour of heated soils will be noticed in other cases.

These results agree with those of Darbishire and Russell recorded in this Journal[1]. These workers had previously shown[2] that the rate at which soil absorbs oxygen is greatly reduced when the soil is nearly

[1] Vol. II. p. 305. [2] Vol. I. p. 260.

sterilised by heating to 120°, but when heated to only 95°, its absorptive power is considerably increased: assuming that oxygen absorption and bacterial contents go hand in hand, this would accord with Hiltner and Störmer's observation that heating to 95°, though it initially reduces the number of bacteria in the soil by 75 per cent., results eventually in an enormous increase in the numbers present. Oxygen absorption, according to Darbishire and Russell, is an index of the fertility of a soil, and, in accordance with this, they found that a number of different plants, other than Leguminosæ, showed an increase in growth ranging from 33 to 380 per cent. when grown in soil heated to 90—95°. The leaves were found to be relatively rich in nitrogen, and the total amount of this element removed from the heated soil by the plant was sometimes three times as much as that removed from unheated soil. It must be remarked, however, that the beneficial effect of heating was found to be increased when a temperature of 120° was employed, and as such a temperature greatly diminishes the oxygen absorption, and nearly, or entirely, sterilises the soil, this fact would appear to exclude either oxygen absorption or bacterial conditions from being the main factors in producing the increased growth. On the other hand, partial sterilisation with antiseptics produced results similar to, though considerably smaller than, those produced by heating to 90—95°, and here, it was argued, the results must depend, at least partially, on the altered bacterial conditions of the soil.

The present experiments add apple trees to the list of plants showing increased growth and nitrogen-assimilation in heated soils. They, however, behaved very unexpectedly in another respect, for they did not start into growth till long after the trees in unheated soil. The latter, of which there were seventeen, were planted under a variety of conditions, but they started at the same time, or within one day of each other. Of the nine trees in heated soil, however, only two (two of those in unsifted earth heated to 200°) started on this date, another did not start till late in the summer, and almost died, whilst the remaining six were quite fourteen days behind those in unheated soil. This delay was attributed at first to the altered bacterial conditions of the soil; and the fact that two of the trees were exceptional in their behaviour, lent colour to such an explanation; for chance re-inoculation of the soil was very possible, whereas chance alteration in its chemical character was hardly so.

An examination of the effect of soil conditions on the starting of trees into activity promised to be such a lengthy task, that it seemed

advisable to attempt to obtain some preliminary light on the subject by first examining a phenomenon of an allied character, namely, the germination of seeds.

Preliminary experiments on germination.

In the first series of experiments, the seeds of *Lolium perenne* were sown in Harpenden earth or in "burnt" sand, which had been heated in an air-bath to various temperatures for 2 hours. The seeds were partially sterilised in carbon disulphide vapour, and were sown at a depth of $\frac{1}{8}$ inch from the surface of the soil or sand, which was placed in bottles through which a current of filtered air was occasionally drawn. All possible precautions were taken to render the conditions aseptic. Ten seeds were sown in each bottle, and each experiment was made in duplicate: these were all very concordant, and the mean results only are given in Table II. The relative values are those compared with the results in unheated earth.

TABLE II. *Lolium perenne in earth and sand.*

Treatment	Earth			Sand		
	Germination, %	Incubation, days	Relative weight of plants	Germination, %	Incubation, days	Relative weight of plants
Unheated	65 = 100	11 = 100	100	65 = 100	14 = 130	65
Heated at 60°	35 = 54	19 = 180	3·3	—	—	—
,, 80°	5 = 8	25 = 231	0·8	—	—	—
,, 100°	5 = 8	69 = 639	0·9	0	∞	0
,, 150°	0	∞	0	—	—	—
,, 250°	0	∞	0	45 = 69	15 = 139	2·6
Heated at 250° and reinoculated	55 = 85	23 = 213	10	20 = 46	16 = 143	30
Treated with CS_2	80 = 123	12 = 107	66	—	—	—

It is evident that the heating of the soil has been very detrimental to germination: even when the temperature had been raised to only 60°, the number of seeds germinating was reduced to nearly half, and the time occupied in the germination of these was nearly doubled; whilst heating the soil to 150° prevented germination altogether. Re-inoculation of heated soil, by adding to it a 10 per cent. soil extract instead of water, has almost restored it to the con-

dition of unheated soil: on the other hand, partial sterilisation by carbon disulphide vapour has had little or no effect. The results in sand are similar to those in earth, though less regular, heating to 100° having prevented germination altogether, although the heating to 250° has had comparatively little effect on the germination percentage, and practically none on the incubation period: inoculation with earth extract has had little effect on the germination. The analogy between the behaviour in heated sand and heated earth is greater when the weight of the seedlings is considered, the heating in both cases having resulted in a remarkable diminution of the growth of the plants, amounting, in the case of earth, to twenty-nine thirtieths at 60°, and to more than ninety-nine hundredths at 80° and 100°. Inoculation with soil extract has also produced analogous results with earth and sand.

In two respects these results appear to be in opposition to those of Darbishire and Russell. These chemists found no effect to be produced on the germination of the seeds by heating the soil[1], and they also found, as already mentioned, that the heating resulted in increased growth. Their experiments, however, were conducted under very different conditions from the present ones, and it will be shown below that the Wye soil used by them exhibits the same retarding effect on germination when heated, as does the Harpenden soil. As to the growth of the plants, the discrepancy may be apparent only: the present results can hardly be accepted as showing what the real behaviour of the plant would be, for, when thus grown in closed bottles, the conditions are quite abnormal, and the plants never reach maturity; in fact, the growth exhibited may, perhaps, be regarded more as extended germination, than as plant-growth. It may be remarked, however, that the roots must have been functioning to a considerable extent, for the ash of the plants grown in unheated soil was 120 times that of the seeds producing them, and, even in the unheated sand, the plant-ash was 100 times that of the seed-ash.

The fact that heating sand had a similar inhibitory effect to that of heating soil, naturally led at first to the conclusion that the question was one of bacterial, and not of chemical, change[2], and it was with this idea that the investigation was pursued.

Method of experimentation.

The further experiments were carried out in sporulating dishes in an incubator at about 23°. Each dish held 15 grams of soil, and

[1] *Loc. cit.* p. 322, and *Nature*, July 4, 1907. [2] *Nature*, June 6, and July 4.

10 seeds were either just buried below the surface of the soil, or, in the later experiments, were simply pressed into it by a platinum disk. The water contents were made up to 23 per cent.

In every case the experiments were made in duplicate, but the means only will be given. The probable error of these, in the case of the times of incubation entered in Table III, as determined by the differences between duplicates, varies according to the temperature to which the soil has been heated, and is:—

Unheated	*Heated to*				
	60°	*80°*	*100°*	*125°*	*150°*
5·4	6·8	8·4	20·7	16·8	39·7 units.
or 5·4	6·2	6·8	14·7	9·1	16·7 per cent.

To obtain uniform results it was found essential that each series of experiments with one kind of seed in soil heated to different temperatures should be made at the same time.

Seed sterilisation.

Carbon disulphide chloroform and ether were each found to be inefficient for sterilising the seeds: formalin—used by driving the vapour from a boiling solution over the seeds, and then leaving these in a tube plugged with wool till all smell had disappeared—was generally effectual, but no means were found for regulating the treatment. Mercuric chloride was finally adopted. The seeds were first treated with ether and evacuated, washed with alcohol, and next with water, and then soaked for 20 minutes in a 0·25 per cent. solution of the chloride, being finally washed thoroughly with sterilised water. There did not appear to be much difference in the sterilising action of a mercuric chloride solution of this strength, and one of half or double the strength; but a 0·5 per cent. solution acting for 40 minutes proved fatal to the seeds. The time occupied in preparing and sowing twenty or thirty dishes with seeds was considerable, and chance re-inoculation could not always be avoided; but some of the seeds were always tested in agar-agar, and occasionally the sterilisation was found to be imperfect, about one seed out of ten giving rise to mould: the mould, however, often appeared only after the lapse of 8 to 15 days, indicating that the germs must have been actually inside the seed. In the following tables, where the test showed this imperfect sterilisation, "sterilised" is placed in brackets, "(St.)"

Germination in agar-agar was obtained with perfectly sterilised seeds of *Lolium italicum*, *Lolium perenne*, clover, spinach and, in one case,

mustard: to these may be added turnips, which were germinated by Dixon under aseptic conditions[1].

Treatment with sterilising agents increases the time required for germination, and (generally) reduces the percentage of seeds germinating, as will be seen by the following values:

	Mustard		*Rye grass*	
Treatment of seeds	°/₀ *germination*	*Time, days*	°/₀ *germination*	*Time, days*
Water	100	2·0	88	4·4
Ether and CS_2	100	2·2	91	4·8
Ditto and $HgCl_2$ 1 : 800...	90	2·4	68	4·5
,, ,, 1 : 400...	90	2·6	91	6·9
,, ,, 1 : 200...	100	2·8	68	6·3

Wheat, rye, mustard, clover, Lolium italicum, Lolium perenne, Dactylis glomerata, and Poa annua. Mean.

	In earth		*In sand*	
	°/₀ *germination*	*Time, relative values*	°/₀ *germination*	*Time, relative values*
Water	100	100	100	100
$HgCl_2$, 1 : 400	80	160	81	221

The soil used.

This was the same as that used in the preliminary experiments, but 4 per cent. of pure calcium carbonate was added to it, and the heating was conducted (for two hours) in closed vessels in an autoclave. The water contents at the time of heating were A, 18·03 p. c., B, 9·28 p. c. and C, 13·5 p. c., in the case of the different batches of soil which had to be prepared.

The water was always made up to 23 per cent. after the seeds were sown; but the results were found to be independent of the amount of water within somewhat wide limits: thus, the behaviour of clover and mustard in soils heated to 60°, 125° and 150° was found to be practically identical, whether these contained 18 or 28 per cent. of water, the average germination percentages being 102 : 100 in the two cases, and the incubation times 104 : 100. As the loss of water during the experiments was found to be only 0·25 per cent. *per diem*, irregularities in the rate of this loss could have no appreciable effect on the results.

The soils heated to 125° and 150° were found to be quite sterile: that heated to 100° decomposed broth much less readily than the soils heated to lower temperatures, and showed a comparative absence of putrefactive bacteria: the soil heated to 80° was less active than that heated to 60°, and this, again, was less active than the unheated soil.

[1] *Trans. Roy. Soc. Dublin*, v (11), p 1.

Results obtained.

Table III contains the results from 21 series of experiments. The first eleven were made with soil sample A, and the rest with B. Six of the first eight were made with seeds which had been sterilised, though, in some cases, not perfectly so; the others are mainly with unsterilised seeds. The last six series consist of three pairs of results, in which sterilised and unsterilised seeds were used, these series being made at the same time. The actual percentage of seeds germinating, and the average time in days required for the germination, are given as regards the unheated soil only: as regards the heated soils, the relative values, compared with those in the unheated soil expressed as 100, are alone given.

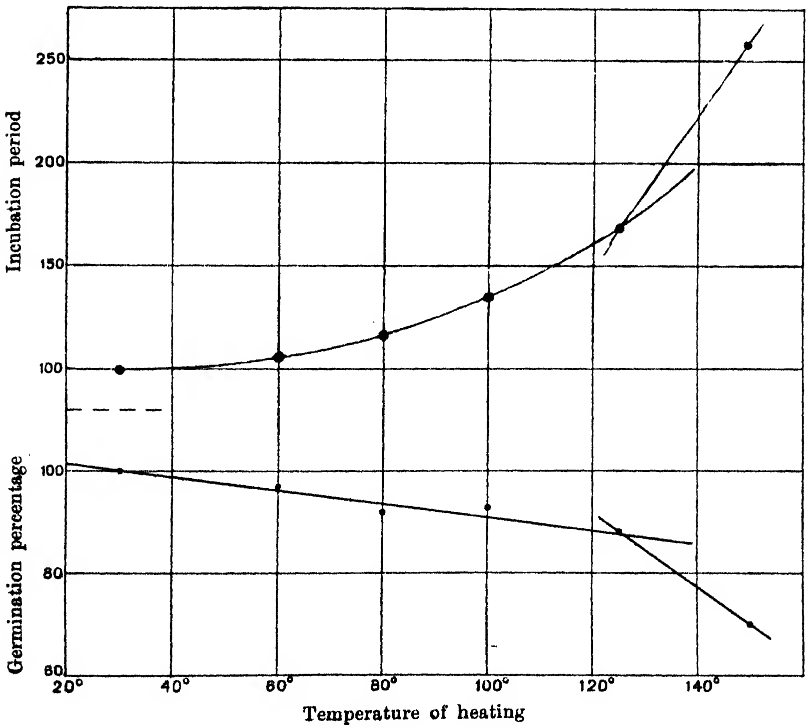
TABLE III. *Percentage of germination and time of incubation of seeds in soil heated to various temperatures.*

Seeds	Percentage germination						Time of incubation					
	Unheated soil, actual	Relative values					Unheated soil, actual	Relative values				
		60°	80°	100°	125°	150°		60°	80°	100°	125°	150°
Mustard (St.)...	100	100	100	95	75	40	2·4	94	99	107	270	574
Lol. ital. St. ...	60	100	150	125	108	75	6·7	99	93	134	167	206
Rye (St.).........	50	170	120	130	120	100	2·0	145	158	235	388	245
Clover St.	90	111	106	95	61	17	2·1	102	117	180	207	402
Lol. peren. St.	15	(167	300	367	233	233)	7·0	107	129	136	100	146
Dact. glom.......	20	175	50	100	75	25	15·0	64	113	90	123	80
Poa annua	55	9	36	82	27	56	5·6	101	171	124	149	261
Wheat (St.) ...	45	100	78	78	78	22	5·2	73	126	125	125	144
Mustard	100	—	100	—	95	80	2·4	—	86	—	194	557
Clover	95	—	100	—	90	63	2·1	—	162	—	169	310
Rye	85	—	100	—	94	106	2·0	—	106	—	108	130
Clover	100	100	100	95	80	85	1·8	103	117	133	150	278
Lol. ital.........	65	77	115	85	145	77	3·6	90	136	133	146	428
Rye	85	118	106	118	112	106	2·5	134	86	94	146	174
Wheat	100	100	95	100	100	95	4·9	94	87	99	106	142
Fest. prat. (St.)	95	84	95	100	84	95	4·1	115	122	143	149	207
,, ,, ...	90	106	94	100	100	100	3·6	110	103	117	143	147
Spinach St. ...	35	72	130	126	163	140	5·3	72	130	126	163	140
,, ...	100	100	100	100	85	95	4·5	107	107	151	159	168
Fest. ov. (St.)	35	57	29	43	29	14	9·3	108	119	132	141	173
,, ,, ...	35	71	29	14	43	0	8·0	144	106	125	137	—
Mean (relative)	100	97	92	93	88	70	100	106	117	135	169	260

In spite of numerous irregularities with individual series, it is clear that, on the whole, the time of incubation increases considerably with an increase in the temperature to which the soil has been heated: at the same time the percentage of seeds germinating decreases with this heating, though the irregularities in this case are considerable, and there are several instances which indicate that heating the soil to the lower temperatures (60° or 80°) has actually increased the germination.

The general features of the results are evidently the same with both samples of soil, and the differences observed in different series appear to be due mainly to the differences in the behaviour of the seeds, some (*e.g.* mustard) being evidently more affected by the heating of the soil than others (*e.g.* rye). The last six series, as well as others, show also that the results are practically the same whether the seeds have been sterilised or not: the mean differences in the times of incubation of the sterilised and unsterilised seeds in these series being:

60°	*80°*	*100°*	*125°*	*150°*
+22	−18	−3	−5	−16



The values in the table, being entered in chronological order, afford evidence that the heated soil preserves its deleterious properties on keeping, at any rate at a winter temperature; for in many of the above cases several months had elapsed between the making of the corresponding series.

In spite of irregularities in the individual series, the mean results are remarkably regular, and, when plotted out, produce the figures shown in the diagram on page 419. (The germination percentages in the case of *Lolium perenne* have been omitted from the mean, as the total germination was only 15 per cent., and the results were proportionately uncertain.) These, in the case of the germination percentages, lie sensibly on a straight line up to 125°, and the incubation times lie very closely on a bent lath curve up to this same temperature. It would, however, require fuller data to warrant the conclusion that the apparent alteration in the action at about 125° is a reality. The "unheated" soil was probably heated to about 30° during its drying in air, and the results with it have been entered for this temperature.

Results with sand.

Before discussing these results, some similar experiments with sand may be described. The sample of sand used in the preliminary experiments had been exhausted, and two other samples were used in the experiments entered in Table IV. The first was a mixture of "burnt"

TABLE IV. *Percentage of germination and time of incubation of seeds in sand heated to various temperatures. Relative values, those in unheated sand being* 100.

Seeds	Percentage germination					Time of incubation				
	60°	80°	100°	125°	150°	60°	80°	100°	125°	150°
Mustard (St.)	100	95	100	95	100	110	93	90	71	88
Lolium italicum St.	94	94	88	94	81	75	79	71	63	93
Rye (St.)	127	155	127	130	127	96	109	81	85	76
Dactylis glomerata	(0	33	167	33	67)	—	100	101	100	123
Mustard (St.)	—	—	100	—	100	—	—	105	—	110
Clover St.	—	—	90	—	105	—	—	108	—	81
Lolium perenne St.	—	—	80	—	100	—	—	105	—	87
Poa annua	—	—	110	—	90	—	—	101	—	101
Mean	107	115	99	106	100	94	95	100	80	95

sand with coarse and fine Leighton sand; the other (used in the last four series entered) was some fine Leighton sand which had been buried in earth for twelve months, so that it should become impregnated as far as possible with soil bacteria.

The results as will be seen are purely negative. Both the means of the germination values (from which those with *Dactylis glomerata* are omitted, owing to the small number of seeds which started), and the means of the incubation times, are all very close to 100—the value in unheated sand—and show no tendency to vary in any definite direction. Whatever the explanation, therefore, of the results with sand in the preliminary experiments may be, it is clear that those results were due to some peculiarity of the particular sample of sand used: no doubt, it must have been accidentally contaminated with sufficient organic matter to make it behave like ordinary soil (see p. 423).

The bacterial explanation.

Summarising the evidence for or against the view that bacterial conditions are factors in the germination of seeds, we have:

First, the fact that many seeds will germinate under aseptic conditions in a sterile medium (p. 417), and that, therefore, bacteria cannot always be necessary factors in the case. Secondly, taking the results with heated soils, the effect of heating on germination is evident at temperatures as low as 60°, probably, even 30°, and is also progressive with the temperature, certainly up to 150°, whereas the bacterial conditions of the soil cease to be affected by any heating above 125°, all bacteria having been killed at that temperature. Thirdly, sand, even when thoroughly inoculated with soil bacteria, behaves in the same way towards germination whether it is heated or not.

Lastly, some experiments were made to see whether the results would be affected by re-inoculating previously sterilised seeds. Seeds of rye-grass and mustard were first sterilised with mercuric chloride, and then half of them were soaked for an hour in a decoction of similar, but unsterilised, seeds; the other half being soaked in sterile water for a like time. Each lot of seeds was then sown, both in unheated earth and in heated sand. The incubation periods of the re-inoculated seeds as compared with those of the sterile seeds (= 100) were

Rye grass	In earth	69
	In sand	114
Mustard	In earth	104
	In sand	100
	Mean	97

thus showing that the re-inoculation had had no effect.

The chemical explanation.

A bacterial explanation of the facts being thus put out of court, a chemical explanation must naturally be sought. That soil on being heated, even to 100°, undergoes chemical change, is well known, and this change, as evidenced by the increasing depth of colour of the aqueous extract, increases at higher temperatures. No acid, however, appears to be produced, at any rate when the soil is heated in a moderately moist condition, and with an excess of chalk in closed vessels, as was the case in the present work.

The aqueous extracts of the soils were investigated as regards the amount of organic and mineral matter contained in them, and also as regards their nitrogen contents. The difference between the weights of the residue dried at 100° and after ignition is entered as organic matter, but it is probable—from the results obtained with some soil which had been heated to redness (p. 425)—that it embraces also a certain amount of constitutional water retained at 100°. For the nitrogen determinations, I am again indebted to Dr E. J. Russell. The results are given in Table V, soil sample B being used for the determination of extract, and sample C for that of the nitrogen.

TABLE V. *Soluble matter in soils heated to different temperatures.*

Temperature of heating	Soluble matter				M/I	O/I	N/I
	Mineral	Organic	Nitrogen	Organic %			
Unheated	·266	·315	·00402	54	—	—	—
60°	·254	·340	·00397	57	—	—	—
80°	—	—	·00425	—	—	—	—
100°	·311	·413	·00796	57	·0013	·0028	·00011
125°	·312	·458	·01553	59	·0007	·0021	·00017
150°	·423	·845	·02921	67	·0010	·0033	·00016

The heating increases the soluble mineral matter, and still more so the soluble organic matter, especially above 125°, the ratio of the two being given in the column headed "Organic, %." The nitrogen contents, also, increase with the temperature, the seeming irregularity at 60° being within the limits of experimental error.

To ascertain whether any direct connexion can be traced between the composition of the soil and the germination results, the increase in the soluble soil constituents has been divided by the increase in the times of incubation as given in Table III. The values obtained by

treating the mineral, organic and nitrogen contents in this way are given in the columns headed M/I, O/I and N/I respectively. In the first two of these, an accumulated error of 2 milligrams in the weighings (independent of any error in the incubation values) would make a difference of ·0002 in the value at 150°, ·0007 in that at 100° and ·004 in that at 60°: this precludes the possibility of attaching any importance to the values at 60° in either case, and, indeed, to any of the values for M/I: it is impossible, therefore, to say how far the incubation results are dependent on the increase in the soluble mineral contents of the soil. As regards O/I the values from 100° to 150° are certainly constant within the limits of experimental error. With the nitrogen values, the experimental error would probably make a difference of at least ·00004 in the value at 100°, and ·000015 in that at 150°, so that here, again, we have a constant well within the limits of experimental error: at 80° and 60° the experimental error is greater than the differences to be measured.

These results, therefore, justify the conclusion that the effect on the germination of seeds produced by heating a soil is directly proportional to the amount of some organic and nitrogenous compound formed during the heating, and is, therefore, in all probability, a direct consequence of it: whether alteration in the mineral constituents of the soil has also an effect the results so far do not settle, but conclusive evidence on this point will be given below.

Heating soils under various conditions.

Some determinations were made to ascertain how far the amount of this organic substance formed on heating a soil was affected by the proportion of water present during the heating. Three samples of the same lot of soil, dried to different extents by exposure to air, were heated

TABLE VI. *Composition and behaviour of soil heated to* 140° *with different amounts of water.*

Water contents of soil	Contents of extract		Relative times of incubation			
	Mineral	Organic	Mustard	Clover	*Lolium perenne*	Mean
14	·345	·545	88	114	116	106
7	·190	·463	83	104	95	95
2·7	·187	·475	100	100	100	100

at the same time in the autoclave at 140° for 2 hours. The composition of their aqueous extracts (Table VI) indicates that the two samples where there was least water were almost identical, whilst that with the greatest amount gave distinctly more soluble organic matter, and nearly double the amount of soluble mineral matter. The germination results obtained in these soils do not in any of the three cases investigated follow the values for the soluble mineral matter, but the mean results do follow those for the soluble organic matter, though the differences are small. Similar results with soils heated at 150° with different amounts of water will be found in Table VII.

TABLE VII. *Composition and behaviour of soils heated to high temperatures.*

Series I. Mean of results with mustard, clover, *Festuca pratensis*, rye, wheat and *Lolium italicum*.
Series II. Mean of results with mustard and *Festuca pratensis*.
Series III, IV and V with mustard.

Treatment of soil	Contents of extract		Incubation times of seeds				
	Inorganic	Organic	Series I	Series II	Series III	Series IV	Series V
Unheated	(·266)	(·315)	—	55	22	(30)	(30)
150° moist	(·423)	(·845)	—	—	—	167	172
150° dry	·256	·616	100	100	100	100	100
200° ,,	·339	·634	118	—	—	—	—
250° ,,	·267	·493	103	—	—	—	—
350° ,,	·287	·235	—	81	40	—	—
Redness	·240	·070	—	{63} {61}	23	—	—

In some other experiments the question of the effect of the length of time for which the soil was heated, was investigated. Some of the same sample of soil (containing 9 per cent. of water) was heated at 100° for 2, 6 and 12 hours respectively; and the relative incubation periods obtained with these samples were:

	Clover	*Festuca pratensis*	Mean
Heated 2 hrs.......	100	100	100
,, 6 ,,	90	136	113
,, 12 ,,	105	92	99

from which it appears that they all behaved in a similar manner within the limits of experimental error.

If the altered behaviour of soils is due to the organic substances rendered soluble by the heating, a temperature sufficiently high to destroy these substances should result in restoring the soil to its original germination capacity.

For heating above 150°, an air-bath had to be used: this involved a total loss of the water present. The results with soils thus heated are given in Table VII, in which are also inserted in brackets, for the sake of comparison, the results with moist soils heated at 150° in the autoclave, and also with unheated soils, as given in previous tables. The heating (soil C, being used) was for 2 hours at the temperatures named, except with the sample heated to low redness in an open dish, where 1 hour only was allowed. What is entered as soluble "organic" matter in this sample must be chiefly constitutional water retained by the inorganic residue at 100° (see p. 422).

The germination experiments were, unfortunately, not well devised, and several series have had to be pieced together; the results with soil heated to 150° have been taken as the common standard of comparison. The first series, with six different seeds, gives 200° as the temperature of heating which produces the maximum retarding effect, and this agrees with the temperature at which the organic matter rendered soluble is greatest. With higher temperatures, both the retardation and organic matter decrease (series II and III), till, at a red heat, the latter becomes nil, and the incubation time of the seeds is the same, or very nearly the same, as in unheated soil. These results, therefore, afford further strong evidence that it is the organic matter rendered soluble by the heating which is the inhibitory agent as regards germination: they cannot be explained by the variations in the soluble inorganic matter.

The difference in the composition of the soils heated to 150° with and without drying, and the different effect of them on seeds, as shown by the 4th and 5th series, afford additional evidence in the matter, and supplement the results already given in Table VI.

Behaviour of the inhibitory substance in heated soils.

That the extract of a soil which has been heated will impart its inhibitory properties to a soil which has not been heated, is shown by the results in Table VIII, wherein unheated soil was treated with various quantities of an extract obtained by digesting soil which had been heated to 150° with an equal weight of water. Moreover, the retardation of the germination appears to be directly proportional to percentage of inhibitory matter present in the water contained by the soil. The calculated values

are based on this supposition, and they agree within experimental error with the values found in the case of mustard: the differences in the case of *Festuca* are too small to lead to any conclusions.

TABLE VIII. *Effect of treating soil with extract of heated soil.*

Soil used	Time of incubation			
	Mustard		*Festuca pratensis*	
	Found	Calculated	Found	Calculated
15 grs. unheated + 3·5 c.c. water......	100	—	100	—
15 grs. unheated + 3·5 c.c. extract ...	129	123	103	102
15 grs. unheated + 7 c.c. extract......	133	128	105	103
15 grs. heated + 3·5 c.c. water.........	271	—	110	—

On the other hand, unheated soil does not appear to contain anything which will appreciably counteract the inhibitory action of heated soil. Soil which had been heated to 150° was digested for 5 days in the incubator at 23° with extract of unheated soil—this extract being derived from a weight of soil equal to one-sixth of the heated soil in the dishes—and though the incubation period of seeds then sown in it was found to be reduced from 100 to 85 (Table IX), a precisely similar

TABLE IX. *Results with soil digested with water, etc. before sowing.*

Soil used		Treatment of soil					Seeds used
Heated to	Water %	Not digested	Digested with: Extract	Digested with: Lime-water	Digested with: Water	Unheated soil	
150°	26	100	72	69	66	30	Clover
150°	26	100	97	98	104	57	*Festuca*
—	—	*100*	*85*	*84*	*85*	*44*	*Mean*
150°	23	100	—	—	79	28	Mustard
150°	17	100	—	—	116 } 99	30 }	Clover }
150°	26	100	—	—	81 }		
125°	19	100	—	—	72 } 104	56 }	
125°	28	100	—	—	136 }		
60°	20	100	—	—	92 } 89	102 }	
60°	29	100	—	—	86 }		

reduction occurred when lime-water or pure water was added, instead of soil-extract. Had the inhibitory action been entirely destroyed, the incubation period would have been reduced to 44 in these cases. Further experiments on the digestion of other heated soils with various proportions of water are given in the table, and they lead to very variable results; in most cases some reduction of the incubation period has been produced, but the average of the last six entries gives this reduction as only 3 per cent. Probably the destruction of the inhibitory substance is due to a process of oxidation, which is affected by the minutiae of the circumstances attending each different case.

That no appreciable destruction of the inhibitory substance occurs when the soil is kept for several months at low temperatures, and in a moderately dry condition, has already been mentioned (p. 420).

Behaviour of different soils.

It was of importance to ascertain how far the phenomena exhibited by the Harpenden soil were common to other soils. Three soils were selected for the purpose: one from Ridgmont, Beds., which is a fairly fertile soil on the Oxford clay; another from the chalk formation at Wye, Kent—this being the same soil as that used by Darbishire and Russell in their work—; and a third from Millbrook, Beds., where the soil is excessively sandy and poor. The soils were all taken from land under cultivation, as also was the Harpenden soil, although in that case the land had consisted of old pasture previous to 1893. Analyses of these soils are given in Table X, though 4 per cent. of chalk was added

TABLE X. *Analyses of the soils used.*

	Harpenden	Ridgmont	Wye	Millbrook
Nitrogen	0·25	0·28	0·17	0·10
Water and organic matter	6·94	6·30	3·94	2·75
K_2O	0·27	0·60	0·35	0·18
CaO	0·43	1·03	5·6	0·35
Iron oxide and alumina	10·19	—	7·94	5·66
P_2O_5	0·114	0·22	0·143	0·15
Silicates and nitrog. matter	80·8	—	—	90·7
Coarse sand	7	40	21	81

to each of them, except the Wye soil, for the purpose of the present experiments.

The soils were air-dried till they appeared to be in a similar condition of dryness, when they were found to contain, Ridgmont 15·8, Wye 6·9 and Millbrook 4·0 per cent. of water. They were all heated at the same time in the autoclave, and in the germination experiments 5 c.c. of water were added to each 15 grams of the Ridgmont soil, and 4 c.c. to each 15 grams of the others, these quantities seeming to produce the same degree of moistness.

The germination experiments with these soils were all made at the same time, so as to be strictly comparable; and a series with the unheated Harpenden soil was also made, as well as one in which moistened blotting paper was used in the place of soil. The percentage of seeds germinated lead to no definite conclusions, and the mean results only are given in

TABLE XI. *Germination of seeds in soil from different localities.*

Temperature of heating	Germination percentage, relative	Relative times of incubation						
		Wheat	Rye	Clover	Mustard	*Lolium italicum*	*Festuca pratensis*	Mean
				Ridgmont soil				
Unheated	100	100	100	100	100	100	100	100
60°	101	107	243	106	89	107	121	129
100°	100	112	338	100	161	123	133	161
150°	93	114	229	222	200	123	131	170
				Wye soil				
Unheated	100	100	100	100	100	100	100	100
60°	99	102	128	90	145	111	79	114
100°	103	111	139	122	200	116	90	135
150°	105	117	114	157	245	111	90	140
				Millbrook soil				
Unheated	100	100	100	100	100	100	100	100
60°	105	107	139	111	85	106	85	106
100°	101	115	121	107	130	114	111	116
150°	105	105	150	107	200	140	114	135
				Harpenden soil				
Unheated	—	107	120	86	87	85	84	95
				Ridgmont soil				
Unheated	—	100	100	100	100	100	100	100
				Moist paper				
	—	103	126	116	96	98	91	105

Table XI; indeed, the percentage seems to be somewhat greater with the heated than with the unheated soils, the averages being

Unheated	*60°*	*100°*	*150°*
100	101·7	101·3	101·0 p.c.

but the run of these values is similar to that of the same seeds in Harpenden soil, the results from Table III giving

Unheated	*60°*	*100°*	*150°*
100	109	101	83 p.c.

although, when the results with other seeds are included, a diminution with rising temperature is observed throughout (p. 418).

The times of incubation, however, show a fairly steady increase in each case with the temperature of heating, this increase being greatest with the Ridgmont soil, and least with the Millbrook soil, though in all cases it is probably much less than with the Harpenden soil, where the values for the same seeds, according to Table III, would be

Unheated	*60°*	*100°*	*150°*
100	106	137	302 p.c.

A more striking illustration of the effect of heating the soil is obtained by setting out the percentages of seeds germinated within a given time; 24 or 48 hours, according to the different seeds.

Percentage of seeds germinated in 24 or 48 hours.

Soil	*Unheated*	*60°*	*100°*	*150°*
Ridgmont	58	45	33	8 p.c.
Wye	50	43	27	13 p.c.
Millbrook	58	49	36	26 p.c.

In Table XII the values for soils heated to each different temperature are treated separately, the results being expressed in relation to the Millbrook soil, taken as 100.

TABLE XII. *Times of incubation of seeds in different soils, heated and unheated.*

Soil	Times of incubation, relative values					Increase of organic matter by heating to 150°
	Unheated	Heated to 60°	Heated to 100°	Heated to 150°	Average of heated soil	
Ridgmont	88·5	105	112·5	115	110·8	·632
Wye	96·5	102·5	107	99	102·8	·335
Millbrook	100	100	100	100	100	·142

In the last column are entered the percentages of organic matter rendered soluble by heating to 150°, the details on which these last quantities are based being given in Table XIII, together with the incubation periods for the unheated soils, and also the results for the Harpenden soils and those for water (wetted blotting-paper).

From Table XII it is evident that, in the unheated state, the Ridgmont soil is the most favourable for germination, and the Millbrook soil the least so; but that, after heating, this order is exactly reversed, the reversal becoming more and more marked as the temperature of heating is higher. Further, the extent of the unfavourable effect produced by heating is more or less proportional to the amount of organic matter rendered soluble by the heating (last column), proving that what held good for the same soil when heated to different temperatures, holds good also for different soils heated to the same temperatures.

On looking at the more detailed results with the unheated soils (including that from Harpenden) in Table XIII, it is evident that, in their case, the results are the reverse, and that the time of incubation of seeds in them is less in proportion as the amount of soluble organic matter in the soil is greater.

TABLE XIII. *Times of incubation of seeds in unheated soils, and composition of soil extracts.*

Soil	Incubation times, relative	Composition of extract			
		Unheated soils		Soils heated to 150°	
		Organic	Mineral	Organic	Mineral
Harpenden	84·5	·315	·266	·845	·423
Ridgmont	88·5	·167	·128	·799	·250
Wye..............	96·5	·075	·137	·410	·145
Millbrook	100	·078	·075	·220	·093
(Water	93·5	0	0	—	—)

The general conclusion from these results must be, that the soluble organic matter in the soils examined is directly favourable to germination, and the more there is of it present, the more readily do seeds germinate[1]; but that on heating, it, or some of the other organic constituent in

[1] It has also been found, in the case of the last three soils in Table XIII, that this also holds good as regards the formation of adventitious roots by trees.

them, becomes converted into a compound which is inhibitory towards germination, and the richer and more favourable the soil was before heating, the more inhibitory does it become after heating.

The results with water, as will be seen from Table XIII, do not fall quite into line with those with soils: if the organic matter in unheated soils is uniformly favourable to germination, every soil should act more favourably than water alone; but the results with water assign to this latter a place intermediate between the Ridgmont and Wye soils. The strict conclusion from this would be, that, whilst some soils contain matter which renders them favourable to germination, others contain matter which is slightly inhibitory, rendering them less favourable for germination than water alone. This, certainly, is not improbable, in view of the fact that the inhibitory substance appears to be formed at temperatures as low as 30°; but it can hardly be affirmed on the strength of these determinations: for, apart from the fact that the difference on which the conclusion would rest approximates dangerously in magnitude to the experimental error, it is questionable whether the physical conditions in the case of seeds sown *on* moistened paper are sufficiently akin to those of seeds sown *in* moistened soil to warrant any rigorous comparison of the two[1]. Silica would have been a more suitable substance to use than paper.

The question of the existence of a toxic substance in soils is one of considerable importance in view of work recently done in America on this subject. In that case, toxicity towards growth, not towards germination, was investigated, but the two phenomena are, no doubt, intimately connected, and should be studied together. Whether the substance formed on heating soils is toxic towards plant growth as it is towards germination, is an open question at present, for the fact that plant growth is not adversely affected by heating the soil may be due to the substance becoming modified in the damp soil, before the plant reaches the stage of active growth. But the formation of it, whether it becomes altered or not, involves a large increase of soluble nitrogen in the soil, and this in itself may be quite sufficient to account for the increased growth and increased nitrogen assimilation observed.

Looking again at Darbishire and Russell's results in the light of the present work, further doubt must arise as to how far bacteria

[1] In connexion with this point it may be mentioned that germination in water vapour takes place more slowly than in the presence of liquid water. Some seeds placed on a platinum gauze tray suspended over water took 2·37 and 1·07 times as long to germinate, in the case of clover and *Festuca pratensis*, respectively, as they did on wet paper.

have yet been proved to be concerned in the matter. With heated soils, there is an increase of bacterial contents and of the oxygen absorption up to about 95°, followed by a rapid decrease in both, even to the extinction of the former at 120°, and it is clear that the incubation period of seeds cannot be primarily conditioned by either of these circumstances, for it increases continuously throughout this range, and even beyond it up to 200°. On the other hand the increase is directly proportional throughout to the extent of the chemical change brought about by the heating—the conversion of organic and nitrogenous matter into a soluble form—and such proportionality appears to extend even to the extreme limits of a red heat. The increase in the growth of plants follows precisely the increase in the incubation times of seeds, and, equally with the latter, it does not harmonise with the alterations in the oxygen absorption or bacterial conditions of the soil, but it does harmonise with those of the soluble nitrogen contents, although mathematical proportionality has not yet been established[1].

With soils treated with antiseptics we have—with the exception of the increase in soluble nitrogen, which has not been examined—every condition and effect exhibited by soils which have been heated, but in a minor degree, such treated soils being analogous to soils heated to about 60°. For attributing the increased growth in these treated soils, to those very conditions which are evidently inadequate in the case of heated soils, the only ground at present is that the antiseptics used could not have affected the soils chemically. This is a point on which we can scarcely speak with certainty, and the fact should be proved or disproved by direct experiment. The extent of the change sufficient to explain the results would, indeed, be very small. Judging by the values entered in Tables VI and X of Darbishire and Russell's paper, the excess of growth of plants grown in treated soil and in soil heated to 92°·5, is about 29 and 215 per cent., respectively: if the increase in growth follows the same course as the increase in the time of incubation of seeds, as given in the diagram on p. 419, the treated soil would be

[1] The additional nitrogen assimilated by the trees and plants in these experiments can be fully accounted for, so far as can be judged by the very imperfect data available, by the increase in the soluble nitrogen produced by heating. But it does not follow that these two quantities should be identical. Nitrogen is being rendered soluble during the growth of the plant, and if a plant gets a good start early in its life, owing to the richness of soil, or other circumstances, it may assimilate a greater excess of nitrogen than could be accounted for by the excess available in the soil to start with. This is certainly so in the case of trees, where the behaviour throughout life is largely conditioned by the growth when young.

analogous to soil heated to 57°. The chemical alteration at this temperature is too small, it is true, to be determined by analyses such as those quoted in Table V, but there should be no difficulty in increasing their accuracy indefinitely, by increasing the quantities of material taken.

SUMMARY.

Soil which has been heated without drying to temperatures from 60° to 150° behaves unfavourably towards the germination of seeds, the total seeds germinating decreasing (in most cases), and the time necessary for their germination increasing, with the temperature of heating. The average results are sufficiently regular to show that the alteration in the soil must begin at temperatures as low as about 30°.

Sterilised seeds behave in the same way as unsterilised ones, except that the time required for germination is uniformly longer. This is due to an alteration in the seeds by the sterilising agent, and not to the destruction of bacteria, for sterilised seeds do not recover their property of ready germination on being re-inoculated. Various sterilising agents were examined, but mercuric chloride was the only one found to be satisfactory and efficient.

The retarding effect on germination produced by heating the soil cannot be explained by an alteration in the bacterial condition of the soil, for the alteration extends progressively at temperatures beyond that sufficient to destroy all bacteria: moreover, no similar results are obtained with sand, even when this has been thoroughly impregnated with soil-bacteria to start with; also, re-inoculating previously sterilised seeds has no effect on their germination; and many seeds, after sterilisation, will germinate freely in a sterile medium.

By heating the soil, an increase in its soluble constituents occurs, especially in the soluble organic and nitrogenous matter, and the increase in either of these has been found to be directly proportional, within the limits of experimental error, to the increase in the time required for germination. The latter increase appears, therefore, to be due to the formation of a nitrogenous compound in the soil, which is inhibitory towards germination. This compound is sufficiently stable in solution for an extract of heated soil to affect an unheated soil when it is added to this latter: it also does not seem to be destroyed when the soil containing it is kept at a low atmospheric temperature for some months; but at a higher temperature, and in the presence of sufficient moisture,

it generally loses some of its inhibitory properties, probably through oxidation.

The inhibitory substance is not of an acid nature.

Soils from different localities favour the germination of seeds to different extents, the extent varying (in the three instances examined) directly with the amount of soluble organic matter in the soil: but, on heating these soils to the same temperatures, their action is exactly reversed, the soil richer in soluble organic matter forming, on heating, a larger proportion of inhibitory matter, and becoming, therefore, less favourable to germination.

The experiments do not settle definitely whether any of the inhibitory substance is ever present in soils before artificial heating, but it seems probable that such is the case.

The temperature of heating at which the inhibitory substance is formed in greatest quantity is about 200°: it diminishes in amount as the temperature is further raised, till it disappears entirely at a low red heat, such burnt soil behaving in nearly the same way towards germination as does unheated soil.

The substance which is inhibitory as regards germination need not necessarily be so as regards plant growth, or it may become destroyed before growth becomes active. Its presence results in an increase in the soluble nitrogen in the soil, and this may be the chief, if not the sole, reason of the increase of growth of non-leguminous plants in heated soil. Preliminary experiments with apple-trees have led to similar results, there being a large increase in growth, in leaf-formation, and in the percentage of nitrogen in the leaves, when the trees were grown in heated soil. The increase, also, was greater as the temperature of heating had been higher.

The heated soils, however, behaved towards the starting into growth of the tree in the same way as they did towards the germination of seeds, this starting into growth having been considerably delayed by the heating.

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS.